DISSERTATIONES KINESIOLOGIAE UNIVERSITATIS TARTUENSIS
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ADAPTIVE CHANGES OF MYOSIN ISOFORMS IN RESPONSE TO LONG-TERM STRENGTH TRAINING IN SKELETAL MUSCLE OF MIDDLE-AGED PERSONS

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Venojärvi M., Puhke R., Hämäläinen H., Marniemi J., Rastas M., Rusko H., Nuutila P., Hänninen O., Aunola S. Role of skeletal muscle-fibre regulation glucose metabolism in middle-aged subjects with impaired glucose tolerance during a long-term exercise and dietary intervention. Diabetes, Obesity and Metabolism, 2005, Nov 7(6): 745–754.

ABBREVIATIONS

ATP – adenosine triphosphate
GS – glycogen synthase
GSK-3 – glycogen synthase kinase-3
HSP – heat shock proteins
IGF – insulin like growth factor
IGT – impaired glucose tolerance
MGF – mechano-growth factor
MHC – myosin heavy chain
MLC – myosin light chain
mRNA – messenger ribonucleic acid
RM – repetition maximum
SDS-PAGE – sodium dodecylsulphate polyacrylamide gel electrophoresis

INTRODUCTION

Skeletal muscle tissue forms approximately half of the human body. The main function of skeletal muscle is to perform body locomotion and antigravity function. Skeletal muscle is also the main metabolic organ of the human body, which converts chemical energy to mechanical work.

Skeletal muscle is composed of different types of muscle fibers, which have been classified according their functional, metabolic and morphological properties. The extensive heterogeneity of muscle fibers supports muscle work in different metabolic and mechanical conditions. Muscle fiber composition in skeletal muscle depends on results of genetic and environmental factors, including physical activity.

Myosin is a key contractile protein of striated muscle, which converts chemical energy (ATP) to mechanical work. Three distinct myosin heavy chain isoforms (MHC I, MHC IIa, MHC IIx) and five light chain isoforms (MLC 1s, MLC 2s, MLC1f, MLC 2f, MLC 3) have been found in adult human limb muscles. Although additional MHC isoform genes (MHC Iα, MHC IIb) are represented in human, these MHC isoforms have not been found in limb muscles on the protein level (Weiss *et al*., 1999). MHC profile of single muscle fibers can be used as a molecular marker of muscle fiber classification.

The expression of MHC isoforms is modulated by different physiological conditions, such as increased neuromuscular activity by exercise training. Significant changes on protein level occur only following a long training period. Transition of MHC IIx towards MHC IIa due to specific types of resistance or weight training lasting 8–24 weeks has been indicated in several previous studies (Adams *et al*., 1993; Sharman *et al.,* 2001; Campos *et al*., 2002; Harber *et al*., 2004). A decrease of the proportion of MHC I isoform has been shown to occur after sprint or strength training (Andersen *et al*., 1994; Liu *et al*., 2003b). We have not found any follow-up studies concerning MHC adaptation to long-term (about $\geq$ 1 year) exercise training in healthy people.

MLC adaptation to exercise training has been reported in several studies on laboratory animals (Ingalls *et al*., 1996; Wahrmann *et al*., 2001; Wada *et al*., 2003), but not so extensively in human muscles. Trappe and co-workers (2000, 2001) examined MLC profile during 12 weeks of resistance training in elderly subjects but no significant changes in MLC profile were found. Only in few studies MHC isoforms are assessed by using single muscle fibers and fewer studies have reported parallel expressions of MHC and MLC isoforms in the same muscle fibers (Trappe *et al.*, 2000, 2001, Williamson *et al*., 2000). It has been indicated that the proportion of hybrid fibers decreases during the adaptation to resistance training both in old and young participants (Andersen *et al*., 1994, Williamson *et al*., 2000, 2001).

Human lifestyle has drastically changed during the past century as the share of physical work in daily life has decreased. Sedentary lifestyle is a risk factor

of several chronic diseases, as coronary heart disease, type 2 diabetes and obesity (Simpson *et al*., 2003; Janssen *et al*., 2004; Richardson *et al*., 2004; Strandberg *et al.*, 2004). The effect of physical activity on skeletal muscle is well studied during exercise training, however, less attention has been paid to the lack of physical activity in sedentary persons. Inadequate physical activity is associated with atrophy of muscle fibers and reduced muscle strength (Booth *et al*., 2000; Greelund *et al*., 2003). The loss of muscle mass and function increases the risk of falls and diminishes the level of self-coping in old age. It is possible that physically active lifestyle in middle age can delay age-related changes in old age.

Increased physical activity and changes in daily dietary habits are important factors in the prevention of type 2 diabetes in middle age. Previous studies have shown that lifestyle changes result in significant weight loss and improved glucose tolerance in middle-aged obese subjects with impaired glucose tolerance (Andersen *et al*., 1999; Janssen *et al*., 2004). The knowledge of glucose metabolism in skeletal muscle is mostly based on studies with a short intervention period but Mensink *et al*., (1993) reported that lifestyle changes led to improved glycemic control and enhanced fatty acid utilization in the skeletal muscle following 1-year intervention.

REVIEW OF LITERATURE

1. Diversity of skeletal muscle fibers

1.1. Skeletal muscle fibers classification

Mammals' skeletal muscle is composed of different populations of muscle fibers which vary extensively between species and individuals. Presently, two main histochemical fiber typing methods have been used.

Traditional histochemical muscle fiber typing is based on the mATPase activity after alkaline or acid pre-incubation, distinguishing one slow (I) and two fast type muscle fibers (IIA and IIB) in adult human limb muscles (Brook and Kaiser, 1970). With the advancing of histochemical staining technique, additional fiber subtypes IC, IIC, IIAC and IIAB have been investigated in human skeletal muscles (Ingjer, 1979; Staron *et al*., 1983; Staron, 1997).

According to the activity of either aerobic oxidative or anaerobic glycolytic metabolic enzymes, skeletal muscle fibers are differentiated as slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), and fast-twitch glycolytic (FG) fibers (Peter *et al*., 1972). NADH tetrazolium reductase and succinate dehydrogenase were chosen as reference enzymes for aerobic oxidative metabolism, and glycerolphosphate oxidase was chosen as a reference enzyme for the anaerobic glycolytic pathway. Metabolic properties of fibers do not correlate well with mATPase classification. For example, I type fibers are more oxidative than IIB type fibers but large variability of enzymatic activity in the same fiber type exists (Reichman and Pette, 1982). In addition, the activity of metabolic enzymes depends upon the "training state" of muscle fibers. The half-lives of metabolic enzymes are relatively short and therefore changes in enzymatic activity may rapidly change in response to altered functional demands as exercise training.

There are also several other fiber typing methods as typing according myoglobin content (Krenács *et al*., 1989), Z-line thickness, fiber shortening velocity and others. All muscle fibers classification schemes have distinct limitations and the choice of method depends on the aims of study. MHC profile in a single muscle fiber can be considered as the most suitable marker for muscle adaptation to exercise training.

2. Skeletal muscle myosin

Myosin is the main contractile protein of striated muscle, which converts chemical energy to mechanical work. The native myosin contains one pair of heavy chains (MHC) and two pairs of light chains (MLC).

2.1. Myosin heavy chain

Nine distinct MHC isoforms have been detected in mammalian muscles: MHC β/slow, MHC IIa, MHC IIx, MHC IIb, MHC emb (embryonic), MHC neo (neonatal), MHC α, MHC eo (extraocular) and MHC m (mandibular or masticatory). Only one slow (MHC I) and two fast type MHC isoforms (MHC IIa and MHC IIx) are expressed in human limb muscles. Although additional MHC genes (MHC Iα, MHC IIb) are represented in humans, these MHC isoforms have not been found in limb muscles on the protein level (Weiss *et al*., 1999).

When comparing three methods: mATPase fiber classification, antibodies against MHC isoforms, and probes against their mRNAs, it was established that human IIB fibers do not contain MHC IIb isoform but isoforms similar to the MHC IIx(d) of rat and rabbit (Smerdu *et al*., 1994, Ennion *et al*., 1995). Therefore the fastest human muscle fibers typed by mATPase histochemistry are termed as type IIB but the fastest MHC isoform is termed as type MHC IIx.

2.2. Myosin light chain

Each MHC is associated with a pair of MLC. MLC consists of essential light chain (also named alkali light chain) and regulatory light chain (also named phosphorylable light chain). Essential MLC contains two fast (MLC1f and MLC3) and one slow (MLC1s) isoforms. Regulatory part of MLC includes both slow and fast type MLC isoforms (MLC2s, MLC2f).

Although the MLC profile has been investigated in several previous studies, the final role of MLC is presently poorly understood. Their location near the hinge region of myosin suggests that MLC may be involved in modulating interaction between myosin and actin (Schaub *et al*., 1988). Correspondence between maximum shortening velocity (V_o) and alkali MLC isoform (MLC3) has been reported in studies using laboratory animals (Bottinelli *et al*., 1994; 1995). In few studies where MLC profile and contractile properties of human muscles have been studied, significant correlation has not been found (Larsson and Moss, 1993; Widrick *et al*., 2002).

2.3. Effect of exercise training on the synthesis rate of muscle proteins

The balance between protein synthesis and breakdown determines the quality and quantity of muscle proteins. Quantitative remodelling affects muscle protein mass, and induces skeletal muscle atrophy or hypertrophy. Qualitative modelling of muscle proteins includes the replacement of one protein isoform by the other that is better suited for specific condition.

Distinct muscle proteins have different turnover rates, which are multi-factorial and dependent on their specific half-life. Half-lives of myofibrillar proteins are relatively long (7–10 days of MHC) and therefore changes in myofibrillar proteins and their isoforms occurr relatively slowly. The synthesis rate of muscle proteins is activated even after a single session of exercise training. Fractional synthesis rate of mixed muscle protein increases after a single session of moderate-intensity aerobic exercise (Sheffield-Moore *et al*., 2004) and resistance training (Trappe *et al*., 2004). Muscle protein synthesis elevates rapidly after exercise and remains elevated to 48 hrs (Chesley *et al*., 1992, Trappe *et al*., 2002), whereas in untrained subjects the value of synthesis rate increases considerably more than in trained subjects (Donners and Rennie, 2003). The increased synthesis rate of mixed muscle proteins and MHC have been investigated after exercise training in both young (Chasley *et al*., 1992; Phillips *et al*., 1997) and old participants (Yarasheski, 1993; Hasten, 2000; Balagopal *et al*., 2001).

Similarly with protein synthesis, exercise training activates the breakdown of muscle proteins (Phillips *et al*., 1999). If the synthesis of protein exceeds protein degradation, positive protein balance or, more specifically, muscle hypertrophy may occur. Negative balance of muscle proteins causes muscle atrophy. Muscle protein synthesis decreases with increasing age, beginning with middle age and accelerating with the advancing of age (Yarasheski *et al*., 1993; Balagopal *et al*., 1997, 2000; Hasten *et al*., 2000; Short *et al.*, 2004). The lower synthesis rate of mixed muscle proteins and MHC has been indicated in middle-aged and old subjects more than in young subjects (Balagopal *et al*., 1997). Similarly, in transcript level the MHC mRNA expression (especially MHC IIx and MHC IIa mRNA) decreases at middle age and becomes progressively lower with age (Balagopal *et al*., 2001). The decrease of MHC synthesis correlates with the decline of muscle mass and strength during aging which provides a potential mechanism for sarcopenia (Balagopal *et al*., 1997).

2.4. Expression of MHC isoforms in single muscle fibers

Each MHC isoform has distinct mATPase activity and properties of shortening velocity, therefore MHC profile can be considered as the most suitable marker of fiber type diversity (Bottinelli *et al*., 2001; Pette, 2001). Fibers expressing single MHC profile are called pure fibers and termed as type I, IIa or IIx fibers, respectively. The fibers expressing more than one MHC isoform are called hybrid fibers and termed as type I/IIa and type IIa/x, respectively. The fibers expressing non-nearest-neighbour or multiple MHC isoforms (as type I/IIx and type I/IIa/IIx respectively) have been reported in skeletal muscle of elderly humans (Andersen *et al.*, 1999; Williamson *et al*., 2001).

Functionally, hybrid fibers are allocated as an intermediate step between their next-neighbour pure type fibers: MHC I – MHC I/IIa – MHC IIa – MHC IIa/IIx – MHC IIx. According to the dominant MHC isoform hybrid type fibers can be distinguished as type I/IIa (MHCI > MHC IIa), type IIa/I (MHC IIa > MHCI), type IIa/IIx (MHC IIa > MHC IIx), type IIx/IIa (MHC Iix > MHCIIa).

Electrophoretical and immunological methods have demonstrated that fibers' classification by mATPase activity is based on specific MHC isoforms (Staron and Pette, 1987). Incompatibility exists in fibers where several MHC isoforms are expressed. It has been an object of discussion whether the histochemical reaction determines only the dominant MHC isoform or the co-expression of several MHC isoforms. It has been postulated that routine histochemical mATPase assessment does not reveal minor changes in the composition of muscle fibers, while MHC isoform analysis is more sensitive to adaptive change caused by exercise training.

2.5. MLC profile in single muscle fibers

Fast type fibers from rat and rabbit skeletal muscle contain mainly fast type MLC isoforms (MLC1f, MLC2f, MLC3) and slow type fibers slow type MLC isoforms (MLC1s, MLC2s). There are few studies where the expression of both MHC and MLC isoforms of human skeletal muscle in parallel in the same muscle fibers have been investigated. In human single muscle fibers, differently from small mammals, fast type MLC isoforms are expressed in slow type fibers (MHCI) and slow type MLC isoforms in fast type fibers (MHC IIa and MHC IIx) (Larsson and Moss, 1993; Widrick *et al*., 1996).

3. Skeletal muscle adaptation

Skeletal muscle fibers have a remarkable ability to alter their phenotype under various physiological conditions including hormonal status, mechanical loading, aging and neuromuscular activity by exercise training (Pette and Staron, 2001).

3.1. Role of hormonal status

Several hormones as growth hormone, glycocorticoids, insulin, testosterone and thyroid hormone have an important role in muscle growth, development and maturation. Growth hormone, insulin and testosterone have anabolic influence glycocorticoids have catabolic influence to skeletal muscle, whereas thyroid hormones can affect muscle fibers profile. Hypothyroidism induces fast-to-slow

transition, while hyperthyroidism induces reverse slow-to-fast transition (Caiozzo *et al*., 1998; Canepari *et al*., 1998).

3.2. Role of mechanical loading

The effect of mechanical loading or unloading on skeletal muscle is well studied in laboratory animals but the choice of unloading models is limited in human studies. In studies of laboratory animals, different unloading models as tenotomy, immobilization, hindlimb suspension or microgravity have indicated mainly slow-to-fast MHC transition (Talmadge *et al*., 2000). On the other hand, fast-to-slow MHC transition has been reported in overloaded muscles during compensatory hypertrophy (Pehme *et al*., 2004) and after immobilization in lengthened position of fast muscles (Goldspink, 1999).

In human studies the effect of mechanical loading has been examined in case of spinal core injury, limb immobilization, hindlimb unloading and spaceflight.

During the first several months after spinal core injury adaptive changes in MHC isoforms do not reach the level of statistical significance (Castro *et al*., 1999). After 24-weeks a significant MHC IIa to MHC IIx transition has been reported in spinal core injured patients (Castro *et al*., 1999). Remarkably high percentage of MHC IIx isoform has been reported in long-term paralysed (Gerrits *et al.*, 2003) and spinal cord injured patients (Andersen *et al*., 1996). D`Antona *et al*. (2003) examined the effect of 3.5 month-long immobilization on MHC profile and reported the lower proportion of MHC I and IIa, and higher proportion of MHC IIx in elderly immobilized patients compared to healthy participants of similar age. All this data support the concept of slow-to-fast transformation from MHCI towards MHC IIx.

In healthy participants the unloading effect has been studied using bed rest period. Six weeks of bed rest is not a sufficient stimulus to induce changes in MHC profile in healthy men (Berg *et al*., 1997; Larsson *et al*., 1996). Andersen *et al*. (1999) reported the increase of fibers expressing mRNA for MHC IIx and decrease of fibers expressing mRNA for MHC I, without significant changes on the protein level after 37-day bed rest period in young subjects. Longer than 87-day bed rest period decreases the percentage of MHC I and increases the percentage of hybrid fibers of both *vastus lateralis* and *soleus* muscles and decreases the proportion of MHC IIa fibers in *vastus lateralis* muscle (Gallagher *et al*., 2005).

3.3. Role of exercise training

In previous studies where skeletal muscle adaptation has been examined to exercise training it was shown to it depend on the type frequency and duration of exercise.

Even a single session of heavy-resistance training elevates the concentration of MHC mRNA (Willoughby *et al*., 2002) while endurance training has a potential to affect significantly the concentration of MHC mRNA only after 7-day training period (O'Neill *et al*., 1999). Significant changes on protein level occur only following a long-term treatment. Adaptive changes of MHC isoforms caused by strength or resistance training affect mainly fast type MHC profile. The decrease of MHC IIx and increase of MHC IIa have been reported after different type of resistance or weight training lasting 6–24 weeks (Andersen *et al*., 2000; Sharman *et al.*, 2001; Williamson *et al*., 2001; Campos *et al*., 2002; Liu *et al*., 2003b).

Few studies where adaptation of MHC profile to endurance training have been studied, reported the decrease of MHC IIx isoforms after 12-week training period (Putman *et al*., 2004).

3.4. MHC isoforms transition in single muscle fibers

Muscle fiber transition from one to another type is a multistage process where changes in myosin profiles have an essential role. Using electrostimulation as a model of activation of neuromuscular activity in rabbit skeletal muscle Pette and co-authors have produced the scheme of sequential MHC transition (Pette, 2001). Skeletal muscle MHC isoforms transform one to another in a stepwise manner: MHC I ↔ MHC IIa ↔ MHC IId ↔ MHC IIb. Generally, increased neuromuscular activity tends to induce fast-to-slow transition, whereas decreased neuromuscular activity caused transition in the opposite direction (Pette and Staron, 1997).

In human studies the fast to slow MHC transition have been demonstrated both in muscle sample homogenate and in single muscle fibers. In the majority of studies slow to fast MHC transition is limited only in fast type MHC profile. The decrease of MHC IIx and increase of MHC IIa have been demonstrated after different type of resistance training in muscle homogenate (Adams *et al*., 1993; Sharman *et al*., 2001; Campos *et al*., 2002; Harber *et al*., 2004,) and in single muscle fibers (Parcell *et al.*, 2005). Additional bidirectional MHC transformation from MHC I and MHC IIx to MHC IIa is presently supported with few data (Esbjörnsson *et al.*, 1993; Andersen *et al*., 1994).

4. Age-related changes in skeletal muscle

Muscle development from postnatal period to adult age includes different stages where muscle proteins and their isoforms are differently expressed. Post-maturation aging in humans is associated with the loss of muscle mass and function which begins to occur already after middle age and increases with advancing age (Lindle *et al*., 1997; Jansen *et al*., 2000).

The increased proportion of slow type MHC isoforms with decreased proportion of fast type MHC isoforms is the main result yielded by previous studies with laboratory animals (Pehme *et al*., 2004). Selective loss of fast type motor units is one of the reasons why destructive changes in fast-twitch fibers occur. The decrease in muscle strength (Lindle *et al*., 1997) and in the number of muscle fibers (Lexell *et al*., 1988) has been reported in middle-aged subjects. Similarly, muscle protein synthesis rate decreases with increasing age (Balagopal *et al*., 1997; Hasten *et al*., 2000; Toth *et al*., 2004). High percentage of slow type fibers and MHC isoforms have been reported in elderly people in cross-sectional studies (Trappe *et al*., 2001; Hameed *et al*., 2003). Conflicting data of age-related changes are reported in human skeletal muscles collected during longitudinal studies (Frontera *et al*., 2000).

Obviously the age-related changes in skeletal muscle are caused by several factors as mutations in DNA, hormonal status, oxidative stress, physical activity and individual variability.

4.1. Health implications and aging

The emergence of skeletal muscle fitness following long-term exercise training is associated with enhanced cardiovascular function and skeletal muscle metabolism. Aging is related with a decline in muscle strength, muscle mass and has been linked with decreased muscle fitness (Kell *et al*., 2001). Sedentary lifestyle causes numerous negative health effects, whereas maintaining an active lifestyle is beneficial to the health status. A positive relationship between muscle fitness and health status exists throughout adulthood and this relationship is consistent with higher levels of muscle strength, endurance and flexibility (Kell *et al*., 2001). Muscle fitness allows to maintain an independent lifestyle for a longer period of time. Unfortunately, sarcopenia and increase in fat mass associated with aging may account for the reduced muscle fiber volume, with less of a decrease in muscle cross-sectional area. The loss of muscle fiber contractile proteins may explain why the force per unit cross-sectional area is significantly reduced with aging (Kell *et al*., 2001).

4.2. Role of myogenic growth factors in aging muscle

Transcription for MyoD has been found to be higher in the muscles of older individuals when compared with those of the young (Marsh *et al*., 1997). This may reflect a continued attempt to maintain muscle-specific expression and ameliorate muscle atrophy (Marsh *et al*., 1997). In the muscles of older animals, the myogenic response to a single bout of weightlifting has been shown to be impaired (Tamaki *et al.*, 2000). MyoD mRNA levels a lower in older rats throughout post-exercise period when compared with the young ones. Skeletal muscle MHC isoform expression has been shown to be regulated at the pretranslational level by myogenic regulatory factors. MyoD and myogenin initiate transcription by binding as either homo- or heterodimers to the regulatory region within the promoter of the respective MHC genes (Benezra *et al*., 1990).

MyoD and myogenin regulate both skeletal muscle specification and differentation. The levels of MyoD have been shown to be higher in FT muscles and seem to be associated with the expression of the MHC IIx isoform, whereas myogenin levels have been shown to be higher in slower-contracting muscles and associated with I and IIa MHC isoforms (Hughes *et al.,* 1997). Even a single session of resistance exercise is effective in up-regulating the type I, IIa, and IIx MHC genes and the increased expression of the MHC mRNA isoforms is correlated to increases in mRNA and protein expression of MyoD and myogenin (Willoughby and Nelson, 2002).

Although the mechanism of load-induced muscle hypertrophy are still not fully understood, the increasing evidence suggests a role for locally expressed insulin – like growth factor-1 (IGF–1) in this process (Hameed *et al*., 2003). Two variants of IGF-1 have been shown in human skeletal muscle: IGF 1Ea, the liver-type isoform and mechano-growth factor (MGF), and activity-sensitive local growth factor (Goldspink and Young, 2001). In young subjects the splicing of these isoforms appears to differ in response to a single bout of weihtlifting, with MGF being upregulated and the IGF-1Ea isoform remaining unchanged (Hameed *et al*., 2003). The old subjects who performed exercise with the same relative intensity showed a tendency for an attenuated MGF response. The type of exercise used by Hameed *et al*. (2003), if performed regularly, promotes muscle growth. Probably the MGF response to exercise is selective and associated with muscle damage.

4.3. Effect of resistance training in middle age

Human muscle strength and the ability to develop explosive force and power are known to decrease with increasing age (Toherty, 2003). The decrease in strength can be explained to a large extent by the reduction in muscle mass and

decline in the intensity of daily physical activities, which are mediated by reduction in the size of individual fibers, particulary type II fibers and the loss of fibers (Lexell *et al*., 1988). It has been shown that systematic strength training in older persons of both genders leads to substantial increases in strength performance. The initial increases in strength in elderly people may primarily result from considerable neural adaptation during earlier weeks of training (Häkkinen *et al*., 1998). However, there are few studies on training-induced hypertrophy of individual muscle fiber level in old age, and these results are often conflicting. It seems that type I and II muscle fiber areas are increased during strength training. It has been shown that strength training leads to significant increases in the fiber areas of type II fibers (Häkkinen *et al*., 2001). The individual percentage values for type II fibers correlated significantly with the individual values for maximal strength recorded at pretraining in elderly groups of both genders and remained significant also at posttraining in elderly women (Häkkinen *et al*., 2001).

4.4. Role of skeletal muscle fibers in glucose metabolism in middle-aged subjects

Pathogenesis of type 2 diabetes is still unclear although there is numerous evidence of risk factors, such as obesity and physical inactivity, that are the main nongenetic determinants of the disease. Impaired insulin sensitivity of skeletal muscle is an early sign of the pathogenesis of type 2 diabetes and can be observed years before the onset of overt diabetes (Koistinen and Zierath, 2002). Skeletal muscle is the main tissue for glucose disposal, accounting for up to 80% of all glucose uptakes under insulin-stimulated conditions. Thus, skeletal muscle is a major tissue determining glucose homeostasis (Nuutila *et al*., 1992). The glucose homeostasis depends on several factors, including glucose transport into the muscles. Glucose transport is influenced by the muscle fiber-type composition and the regulation of glycogen synthesis. Previous studies have shown that obese or type 2 diabetic subjects have a higher proportion of type IIB fibers (MHC IIx) in skeletal muscle tissue (Mårin *et al*., 1994; Hickey *et al*., 1995; Tanner *et al*., 2002), and for insulin sensitivity, the muscle fibers follow the older type I > type IIa > type IIb (James *et al*., 1985; Henriksen *et al*., 1990; He *et al*., 2001). Halseth and co-workers (Halseth *et al*., 2001) have shown in rats that in type II fibers glucose uptake under basal conditions whereas under insulin-stimulated conditions delivery of the glucose is the primary limiting factor in type II fibers (Halseth *et al*., 2001).

In type 2 diabetes, glucose oxidation and non-oxidative glucose disposal in skeletal muscle are impaired (Golay *et al*., 1988; Thorburn *et al*., 1990). The major component of the non-oxidative glucose metabolism is glucose storage on glycogen.

Glycogen synthesis is impaired in type 2 diabetes (DeFronzo *et al*., 1987) because of decreased activity of glycogen synthase (GS), which is the rate-limiting enzyme for this synthesis. The GS protein expression, however, is normal in the skeletal muscle of type 2 diabetic subjects (Vetergaard *et al*., 1993). It has been shown that insulin inhibits the action of the glycogen synthase kinase-3 (GSK-3), and this inactivation is associated with phosphorylation of the serine residues Ser21 and Ser9 in GSK-3 isoforms α and β (Nikoulina *et al*., 2002). The protein expression of the GSK-3 is higher in diabetic subjects than in healthy people and GSK-3 regulates negatively GS (Nikoulina *et al*., 2000). GSK-3 is constitutively active in resting cells and is inhibited by several hormones, such as insulin, the endothelial growth factor, and the platelet-derived growth factor (Nikoulina *et al*., 2002). Insulin inactivation of GSK-3 probably occurs through protein kinase B (Akt/PKB) (Cross *et al*., 1995). In the skeletal muscle, Akt are reported to be the same as of those phosphorylated in response to insulin (Cross *et al*., 1995).

5. Unsolved problems

Progress of new technologies in the last century has diminished physical activity of mankind and caused drastic changes in human lifestyle. Sedentary lifestyle is characteristic of a remarkable part in population of developed countries. It has been postulated by Boot *et al.* (2000) that the loss of adequate amount (historically "normal" amount) of physical activity may cause serious health problems. Inadequate physical activity and sedentary lifestyle is associated with increased risk of several chronic diseases as coronary heart disease, obesity, type 2 diabetes and osteoporosis. Therefore, while the effect of physical activity on health has been extensively demonstrated in previous studies, the possible influence of sedentary lifestyle need more attention in future studies.

Muscle myosin is one of the best studied muscle proteins, but despite numerous studies lot of opened problems exist.

Differently from laboratory animals only three distinct MHC isoforms are expressed in human skeletal muscle. The debate about the expression of additional MHC isoforms in human skeletal muscle is still open. MHC Iα and MHC IIb isoforms have been indicated as possible candidates in mRNA but not in protein level. It is possible that the detection of additional MHC isoforms need develop of new more sensitive methods in muscle research.

A paradoxical situation illustrates the adaptation of MHC IIx to physical activity. MHC IIx is the fastest MHC isoform of human skeletal muscle, which proportion decreases after endurance, strength and even sprint training. The increase of MHC IIx has been reported only in condition of inactivity, as bed rest and immobilization. In sport practice is well known that to develop sprint

performance exercise training is more effective than bed rest. This discordant situation needs explanation in the future.

Presently the role of MLC in muscle contraction is unclear. A consensus that has emerged in several previous papers is that the MHC is primary determinant of muscle contraction, while the MLC complement has a modulatory influence on regulating these properties (Lowey *et. al.*, 1993). In human muscle fibers MLC isoform migration has been differently indicated in gel electrophoresis (Trappe *et al*., 2000, 2001; vs Larsson *et al*., 1993). In addition, the existence of MHC/MLC mismatched fibers in human skeletal muscle is still under discussion (Stephenson 2001). Recently Bicer and Reiser (2005) indicated the large species differences in the expression of MLC isoforms of single muscle fibers of laboratory animals. The expression of MLC isoforms in human skeletal muscle fibers, their role in muscle contraction and muscle adaptation need to be discovered in the future.

AIMS OF THE STUDY

The general purpose of this study was to examine the adaptive changes of MHC and MLC isoforms in human *vastus lateralis* muscle in response to long-term strength and power training in previously untrained middle-aged persons.

The specific aims of this study were as follows:

1. to examine the distribution of MHC isoforms in middle-aged sedentary persons;
2. to examine the adaptive changes of MHC and MLC isoforms in muscle sample homogenate and muscle fiber composition to 54-week strength and power training;
3. to examine the expression of MHC and MLC isoforms in the same single muscle fibers of middle-aged persons due to power type strength training for 22 weeks;
4. to investigate the role of MHC isoforms in the regulation of glucose metabolism in middle-aged subjects with impaired glucose tolerance during long-term physical exercise.

MATERIALS AND METHODS

1. Subjects

The total of 151 middle-aged and young persons volunteered to participate in the present study. The participants were recruited from the staff of local schools and companies whose work does not involve physical activity. Their previous physical activity was investigated by a questionnaire and personal interviews. The inclusion criteria of sedentary participants were sedentary lifestyle, absence of participation in recreational activities or exercise programs for several years before the present study. All subjects underwent physical examination for cardiovascular, pulmonary and neurological status, as well as musculoskeletal disorders. All subjects were informed about the design and possible risks of the study and subsequently signed the consent of participation. This study was approved by the Ethical Committee of the Social Insurance Institution of Turku, Finland. The physical characteristics of participants are summarized in Table 1.

Table 1. Physical characteristics of participants (± SD)

Subjects	n	Age (yr)	Height (cm)	Body mass (kg)
Middle-aged sedentary	89	42.3 ± 7.3	179.2 ± 6.6	81.8 ± 13.4
Young sedentary	13	23 ± 2	181.7 ± 5.2	72.3 ± 11.6
Middle-aged sedentary	16	43.8 ± 5.8	179 ± 5	83.1 ± 12.8
Middle-aged sedentary (C group)	6	40.0 ± 8.5	177 ± 4.0	69.7 ± 9.7
Middle-aged physically inactive	22	56.3 ± 6.3		89.5 ± 8.4
Middle-aged sedentary	5	40 ± 2	176 ± 9	89 ± 3

2. Strength and power training program

The subjects were advised to exercise 3 times a week according to a special strength and power training program for a period of 54 weeks. Simultaneously, they continued 0–2 times a week their previous recreational sports activities (including walking, jogging, cycling, skiing, swimming, and ball games, such as volleyball, badminton, tennis, or soccer). The training group carried out 93.5 ± 12.5% (range 75–118%) of the planned sessions for special training in the gym and 134.6 ± 35.2% (range 76–210%) of the recommended amount of training (3 times a week) including both all type of exercise sessions in the planned program and the subjects' spontaneous recreational sports activities. The strength and power training program was individually adjusted to the muscular performance capacity of each subject.

The supervised program started with a lead-in strength-training period of 6 weeks, followed by a basic strength-training period of 4 weeks. The next phase consisted of progressive strength and power training [at 60–75% and 30–85% of 1-RM, respectively] with stretching and elasticity exercises. For the first four weeks of this phase, the subjects exercised twice a week as consecutive training, together with power training sessions once a week as circuit training, after which they had one consecutive and two circuit training sessions a week during the next four weeks. Thereafter, the exercises were changed and the number of repetitions (varying from 4 to 12 rep.), and later on, also the number of sets, was increased. At the beginning of the primary strength and power training phase, three circuits or sets with a pause of 2–3 min inbetween were performed; at the end of the training period the number of circuits was five. During the pauses the subjects performed warm-up (for muscles to be exercised next) and recovery stretching exercises (for muscles just used).

Power-type training and basic strength training were emphasized by turns, and the exercises were focused mainly on the legs. The training group performed 7285 ± 1099 (range 5378–9868) femoral muscle exercises during the whole training period. In addition were performed various trunk and upper body exercises. Special attention was paid to the velocity of muscle contraction in the exercises performed with small loads (power training) in order to induce training effects on the force and velocity characteristics of leg muscles, and especially on the fast-twitch fibers. After three months there was a two-week 'recovery phase' during which the subjects were engaged in recreational outdoor sports 1–2 times per week. During the next 12 weeks (in the spring) the subjects continued the strength and power training program. In summer, the program included interval running, plyometric drills, shot put, discus and javelin throwing, and circuit training.

Before the training intervention started, seven muscle groups were tested by using 30-second repetition tests. Individual training programs with 10 exercises were drawn up on the special training cards for each person in the training group. Later on, 10-second repetition tests or 1-RM were applied to determine the proper progression for training (additional weight and/or number of repetitions). New, individual training programs were given for each phase: the exercises were changed and loads increased according to the program model.

3. Power type strength training program

The power-type strength training consisted of exercises performed with low loads and high velocities. The purpose was to activate muscles to contract with a high or maximal speed for very short duration. The training sessions were supervised by a qualified instructor. The program lasted for 22 weeks, targeted training frequency was three times a week, and each training session lasted for

60 minutes. The training program included three different exercise periods. The first period, i.e. the orientation phase lasted for 6 weeks and consisted of exercises enhancing basic muscle strength and endurance, cardiovascular fitness, and coordination skills. The second period (10 wks) consisted of strength and power-type strength exercises. The exercises were performed with submaximal and maximal velocity demanding muscle strength equalling to ~40% of the maximum. The third exercise period (6 wks) consisted of power-type strength and high-velocity training demanding muscle strength equalling to ~20% of the maximum. Exercise sessions included flexibility exercises, muscle stretching, brisk walking and light jogging. During the main part of the sessions (30 min), 60% of strength, power and velocity exercises were focusing on leg and 40% on trunk muscles. All sessions ended with stretching and relaxation exercises for 15 min. The training program is described in detail in the study of Surakka *et al.* (2004).

4. Intervention program

The subjects belonging to the intervention group visited a nutritionist seven times during the first year and every three months later on. The subjects were individually advised to consume a diet in which >50% of daily energy was derived from carbohydrates and <30% from fat (less than 10% from saturated fat). Further, the daily intake of cholesterol did not exceed 300 mg/day, and the daily intake of protein was approximately 1.0 g/kg and that of dietary fiber 30 g/day at minimum (Eriksson *et al*., 1999; Tuomilehto *et al*., 2001).

The subjects in the intervention group were individually guided to increase their physical activity, and later on, to participate both in regular resistance training in gym and in aerobic exercise. The subjects were advised to exercise with moderate-to-intensive effort for at least 30 min per session and three to four times a week. During the first 6 months, the intervention was focused on dietary counselling, aimed at encouraging the subjects to change their eating habits. Thereafter, the supervised exercise training twice a week was added to the intervention program. In the beginning, the supervised program consisted of brisk walking, gymnastics and stretching exercises, then was included circuit training without weights, and after one month, also basic muscular strength training with weights equalling to 20–40% of 1 repetition maximum (RM). Later on, both strength exercises and power exercises with heavier weights (50–70% and 30–50% of 1RM, respectively) were included for the purpose of increasing the muscle mass and training the fast twitch fibers. In the beginning, the number of circuits was three and later on four, depending on the participant's physical fitness level. The number of repetitions in a set was 10–15 in the beginning, later on it varied with the type of training. The supervised training was progressive and individually designed, consisting mostly of

strength and power training, interrupted by spinning exercises and aerobic gymnastic exercises. Power-type strength training at gym was performed as circuit training.

5. Anaerobic working capacity

The maximal anaerobic power of leg muscles was measured using a bicycle ergometry modification of maximal anaerobic power test on a treadmill (Rusko *et al*., 1993). In this intermittently progressive test, the loading consisted of 5–10 successive 20-s cycling periods with a pause of 100 s between the periods. The test was continued until the subject could not keep the correct predetermined pace, which was 90 revolutions/min for men younger than 40 years and 86 revolutions/ min for men older than 40 years. The increment in work rate for each consecutive cycling period was adjusted to increase the oxygen uptake by 5–6 $ml \cdot kg^{-1} \cdot min^{-1}$.

6. Explosive force

The explosive force of leg muscles was measured as vertical jumping height. The jumping was performed from a static leg position with the knee angle of 100° and without a countermovement. The hands were held on the hips. Contact mat and reaction time equipment (Newtest powertimer®, Finland) were used to measure the off-the-ground time from which the jumping height was calculated (Bosco *et al*., 1983).

7. Muscle biopsies

Duplicate muscle biopsies of 50–100 mg were taken under local anaesthesia from the left *vastus lateralis* muscle using the percutaneous conchotome technique at the beginning and at the end of the training period. The muscle samples were divided into two parts. One part was mounted in embedding medium (OCT compound, Miels Tissue Tek), frozen in isopentane precooled with liquid nitrogen and stored at –70 °C for later histochemical analysis. The other part was frozen in liquid nitrogen and stored at –70 °C for further analysis of myosin isoforms.

8. Histochemical analysis

Muscle fibers were stained and classified by traditional myofibrillar ATPase activity assessment (Brooke and Kaiser, 1970; Staron *et al*., 1983). Serial sections (10 μm) were cut at –20°C. According to the lability to the acid (pH 4.3 and 4.6) and alkaline (pH 10.3) pre-incubations, the fibers were classified as either type I, IIA and IIB. On the average 534 (range 365–996) fibers were classified in each sample.

9. Separation of myofibrillar proteins

Muscle samples were pulverized in liquid nitrogen and homogenised in phosphate buffer according to Sugiura and Murakami (1990). After extracting for 15 min on ice, the homogenate was centrifuged at 11 000g for 10 min. The supernatant fraction was diluted 1:1 (vol/vol) with glycerol and stored at –20°C. The protein concentration of the homogenates was determined by using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, U.S.A.). Aliquots of protein or a fragment of single fiber fragment were incubated for 10 min at 65°C in lysis buffer containing 10% (v/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% SDS, 0.05% bromphenole blue, 62.5 mM TRIS-HCl pH 6.8.

10. MHC isoform`s separation

The MHC isoform composition was separated by 5–8% gradient SDS-PAGE gel system of muscle homogenate or by 7.2% SDS-PAGE gel electrophoresis of muscle homogenate and single muscle fiber. We used a Bio-Rad Protean II Xi vertical slab gel system.

Electrophoresis lasted 24 h at 120 V and 10°C. The gels were silver stained and protein bands were identified on the basis of marker proteins, Western blot analysis and reports in the literature. To quantify the proportion of distinct MHC isoforms we used computer-based image analysis system and software (Image Master 1D Elite, Amersham Pharmacia Biotech). The example of electrophoretical separation of MHC isoforms from m. *vastus lateralis* is presented in Figure 1.

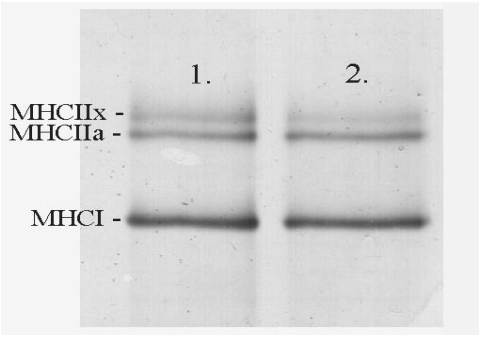


Figure 1. Example of a silver stained SDS-PAGE gel for the identification of MHC isoforms from muscle sample homogenate of m. *vastus lateralis*. Lane 1: from middle-aged sedentary subject. Lane 2: same middle-aged subject after nine months of physical exercise period.

11. Western blot analysis

After electrophoresis, the MHC isoforms were transferred to polyvinyldenedifluoride transfer (PVDFT) membrane (Polyscreen^{TM}, Biotechnology Systems, NEN Research Products, Du Pont) in transfer buffer (20% methanol in running buffer with 0.037% SDS) for 3h at 44 V, 380mA at 5°C. Blots were blocked for 1h at room temperature with 3% bovine serum albumin in phosphate buffering saline-TWEEN-20 (PBS-T-20). The membrane was then washed with PBS-T-20 and incubated with a solution containing primary antibody against slow-type MHC (clone WB MHCs) or against fast type MHC isoforms (clone WB MHCf) from Novocastra Laboratories (Newcastle upon Tyne, UK). Subsequently, the membrane was washed and incubated with a solution containing the secondary antibody (antimouse immunoglobulin-G). The bands were visualized by using enhanced chemoluminescence technique according ECL^{TM} Western blotting protocol (Amersham Biosciences Corp., Piscataway, NJ, USA). The examples of Western blot analysis are presented in Figure 2.

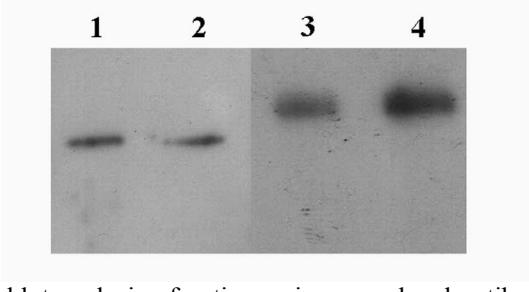


Figure 2. Western blot analysis of anti-myosin monoclonal antibodies. Lane 1 and 2 show immunoreactivity to slow type MHC isoforms; lane 3 and 4 show immunoreactivity to fast type MHC isoforms.

12. MLC isoforms separation

The MLC isoforms were separated by 12.5% one-dimensional SDS PAGE gel system according to Laemmli (1970), except that the glycerol content in the separating gel was 10%. 10μg myofibrillar protein sample was loaded on 1mm thick gel per well. Electrophoresis was carried out at a constant current (30mA) using mini-Protean II Bio-Rad Electrophoresis Cell. The gels were Coomassie blue R-250 or silver stained. The positions of MLC isoforms on the gel were identified by their apparent molecular masses compared with protein mobility of the kaleidoscope pre-stained standard marker proteins (Bio-Rad) and/or compared with protein mobility on the 12.5% SDS-PAGE one and two-dimensional electrophoresis.

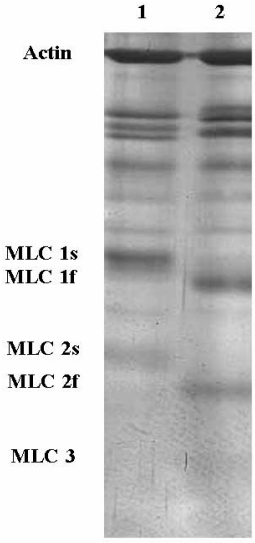


Figure 3. Example of a 12.5% SDS-PAGE analysis of MLC isoforms in single muscle fibers of middle-aged men. Lane 1 represents the MLC profile of MHC I type fiber. Lane 2 represents the MLC profile of MHC IIx type fiber.

13. Two-dimensional electrophoresis

The part of skinned fiber was solubilised in a 30 μl solution containing 9.5 M urea, 2% IGEPAL and 5% mercaptoethanol. Electrophoresis in the first dimension was performed in glass capillaries according to O´Farell (1975), by using 1.6% (pH 5–7%) and 0.4% (pH 3–10) ampholines (Servalyt) in 4.2% polyacrylamide gel using BIORAD, MINI-PROTEAN II 2-D Cell. Electrophoresis was first run for 30 min at 100V, then 1h at 300V and 1h at 400V.

Sepatation in the second dimension was carried out for 4 h at 30 mA in a minigel chamber on a 1.5mm thick 15% separating gel and a 3% stacking gel (Wada and Pette, 1993). The gels were silver stained according to Oakley (Oakley *et al*., 1980).

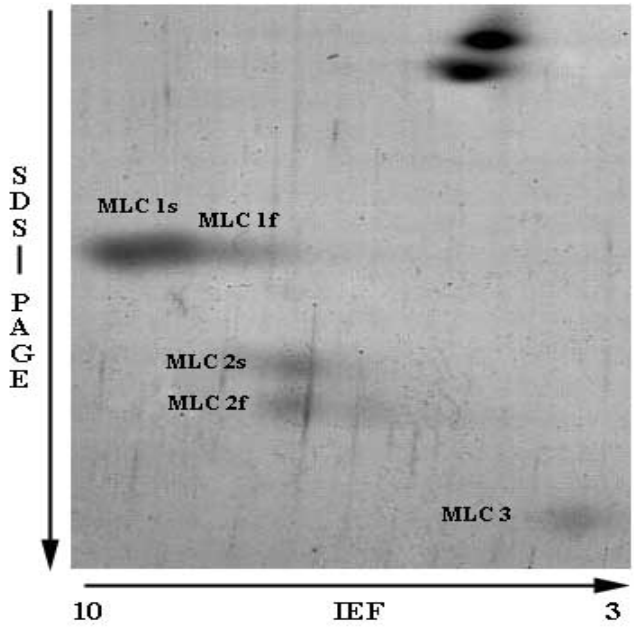


Figure 4. Example of a two-dimensional (2-D) electrophoresis for identification of MLC isoforms in MHC IIa type single muscle fiber.

14. Single fiber dissection

Single fibers were isolated under a stereomicroscope and immersed in skinning solution (150mM potassium propionate, 5mM KH_2PO_4, 5 mM magnesium acetate, 3mM Na_2ATP, 5mM EGTA, 1mM dithiothreitol, pH 7,0) at 12–15°C. The skinned fiber was cut into a two parts; one part for MHC and MLC isoform determination using SDS-PAGE electrophoresis, and the second part for two-dimensional electrophoresis.

15. Identification of single fiber types

According to the MHC isoform's profile was determined the type of muscle fibers. Pure type fibers expressed single MHC isoform profile and termed as type MHC I, MHC IIa or MHC IIx. Fibers containing hybrid MHC profile were quantified densitometrically, and determined by their dominant MHC isoform: type MHC I/IIa, MHC IIa/I, MHC IIa/x, MHC IIax, MHC IIx/a. For example: in type IIax fibers the dominant MHC isoforms does not exist, in type IIa/x fibers the MHCIIa isoforms dominate when their proportion extends from 66.6% to 99.9%. The determination of hybrid fibers are presented in Table 2.

Table 2. Determination of the type of single muscle fibers based on the profile of MHC isoforms

Fiber type	Proportion of MHC isoforms
MHC I	MHC I 100%
MHC I/IIa	MHC I 51–99%, MHC IIa 1–49%
MHC IIa/I	MHC IIa 51–99%, MHC I 1–49%
MHC IIa	MHC IIa 100%
MHC IIa/x	MHC IIa 66.7 –99%, MHC IIx 1– 33.3%
MHC IIax	MHC IIa 33.4 – 66.6%, MHC IIx 33.4–66.6%
MHC IIa/x	MHC IIX 66.7 –99%, MHC IIa 1-33.3%
MHC IIx	MHC IIx 100%

16. Statistical analysis

The data of the present study is presented as group mean (± SD). The differences between middle-aged and young sedentary groups were tested for significance by (I). Differences between the variable means of the training and control groups were assessed using Kruskal-Wallis test, and changes within groups using Wilcoxon' matched-pairs signed-ranks test or Student`s t-test. Pearson's correlation coefficients or Spearman's rank correlation coefficients were calculated to evaluate the associations between the variables and their changes. If it was necessary for Pearson' correlation coefficients, the logarithmic transformations were applied to correct skewed data distributions. The distribution of MHC isoforms in groups was tested using Kolmogorov-Smirnov test. Differences were considered significant at $p < 0.05$.

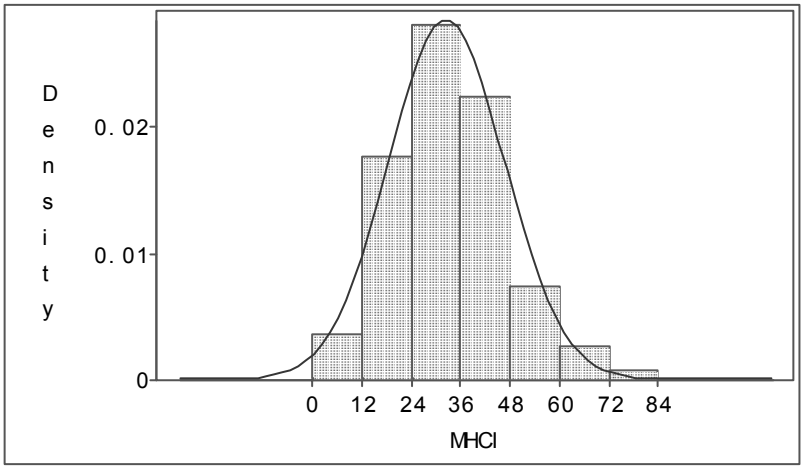
Logical correlation coefficients are presented for evaluating the reliability and accuracy of electrophoretic MLC analyses.

RESULTS

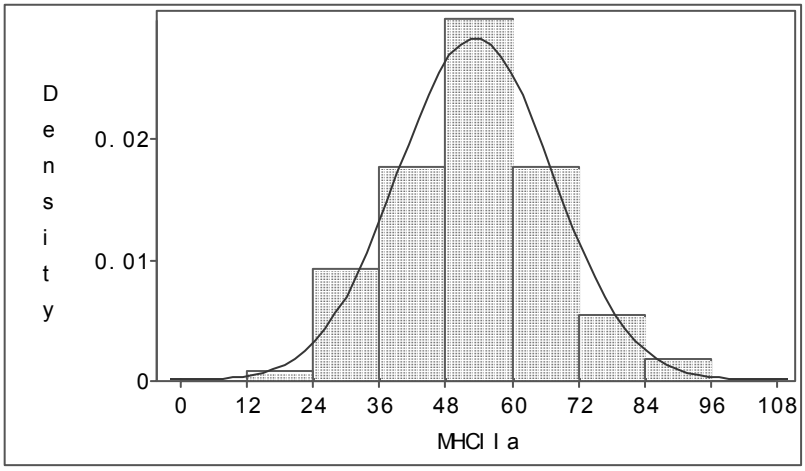
1. The profile and distribution of myosin heavy chain isoforms in middle-aged sedentary persons

MHC profile and distribution were analysed in 89 middle-aged sedentary persons. Large heterogeneity of MHC isoforms was observed in middle-aged participants. The proportion of MHC I varied from 9% to 74%. The variation of MHC I and MHC IIa isoforms in middle-aged sedentary persons demonstrated normal distribution according to Kolmogorov-Smirnov test (Δ=0.058, P>0.15) (Fig. 5). The expression of MHC IIx was observed in 79 participants, varying from 0.8% to 41.7%. The distribution of MHC IIx was out of the normal distribution. Distributions of MHC isoform in middle-aged sedentary participants are presented in Figure 5.

A



B



C

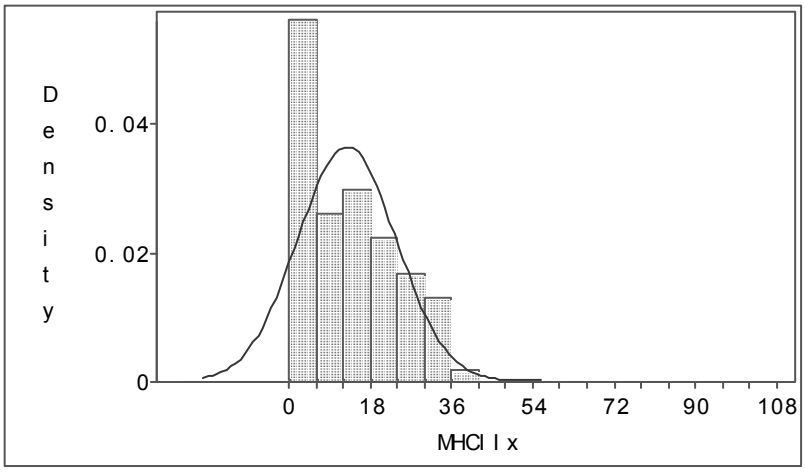


Figure 5. Distribution of MHC isoforms in middle-aged sedentary group (n = 89)
A – Distribution of MHC I isoforms
B – Distribution of MHC IIa isoforms
C – Distribution of MHC IIx isoforms

Comparisons of MHC profile between middle-aged sedentary and young participants does not reveal significant differences. The percentage of MHC isoforms of different groups is demonstrated in Figure 6.

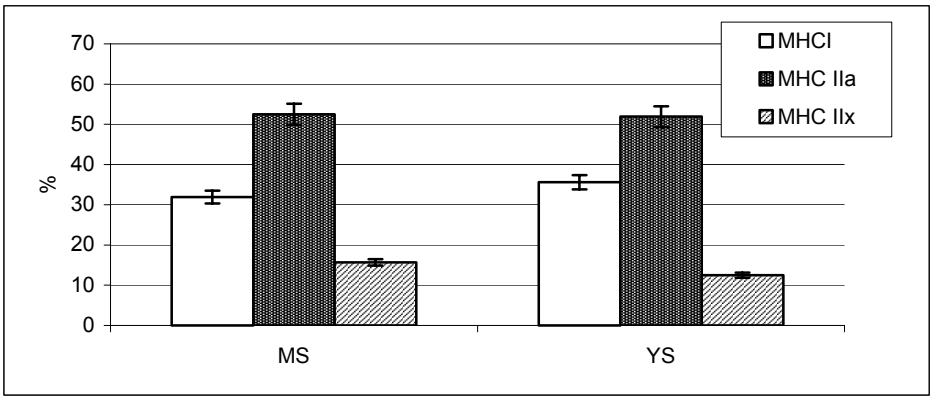


Figure 6. The proportion of MHC isoforms in *vastus lateralis* muscle in sedentary persons

MS – middle-aged sedentary group (n = 89)
YS – young sedentary group (n = 13)

2. Adaptive changes of myosin heavy and light chain isoforms in muscle homogenate during long-term strength and power training

2.1 Anaerobic power and maximal jumping height

The mean values of maximal anaerobic cycling power and maximal jumping height at baseline and after the 54 weeks of strength and power training are presented in Table 3. During the training period, maximal anaerobic cycling power increased by 64 W ($p < 0.001$) and the maximal jumping height by 1.5 cm ($p < 0.05$) in the training group, but no significant changes were found in the control group (Table 3). However, the group by time effect was not significant. In the training group, the increase of the maximal jumping height correlated with the number of strength and power training sessions ($r = 0.56$; $p < 0.05$).

Table 3. Effects of a 54-week strength and power training program on the maximal anaerobic cycling power and maximal jumping height and corresponding work. Values are means (± SD)

	Maximal anaerobic cycling		Maximal vertical jumping			
	power (W)		height (cm)		work during jump (J)	
	Before	After	Before	After	Before	After
Training group (n=16)	538 ± 78	602 ± 87***	32.8 ± 4.3	34.3 ± 5.2*	267 ± 52	281 ± 63 **
Control group (n=6)	474 ± 138)	512 ± 142)	30.3 ± 5.7	28.7 ± 4.9	213 ± 59	204 ± 41

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in comparison with the pre-training period

2.2. Histochemistry

Before the training period, fiber type distributions analysed by histochemical ATPase method were similar in the TR and C groups. After 54 weeks of strength and power training program significant changes in muscle fiber composition were not observed (Table 4).

Table 4. Fiber type (ATPase staining) composition (%) both before and after the 54 weeks of strength and power training in the training and control groups. Values are means ± SD

	Fiber type composition (%)					
	before			after		
Groups	Type I	Type IIA	Type IIB	Type I	Type IIA	Type IIB
Training group (n=16)	42.1 ± 14.4	31.6 ± 9.8	36.3 ± 7.8	40.3 ± 8.9	36.6 ± 10.2	23.1 ± 10.7
Control group (n=6)	39.2 ± 15.3	32.7 ± 11.7	28.3 ± 18.4	43.5 ± 10.3	36.5 ± 5.6	20.0 ± 8.7

2.3. Changes in MHC isoform composition

At baseline, training and control groups differed in the MHC isoforms distribution: the proportion of MHC IIa was 17.6%-units higher and MHC IIx was 11.3%-units lower in the control group ($p < 0.05$ for both; Table 5). After 54 weeks of strength and power training the proportion of MHC I isoform remained the same (Table 5). The change of the proportion of MHC IIa isoform from 52.6 ± 12.2% to 59.4 ± 11.6% did not reach statistical significance ($p = 0.070$ for group by time; within the training group $p = 0.061$) and neither did change the proportion of MHC IIx isoform from 18.1 ± 11.4% to

11.1 ± 9.1% ($p = 0.104$ for group by time; within training group $p = 0.032$) (Table 5). The degree of change of MHC IIx isoform correlated with the earlier recreational sports activity during adult age ($r = 0.61$; $p < 0.05$). No changes in MHC isoforms were found in the control group.

Table 5. Proportion of MHC isoforms (%) before and after 54-week strength and power training program in the training and control groups. Values are means (± SD)

	MHC isoforms (%)					
	Before			After		
Groups	**MHC I**	**MHC IIa**	**MHC IIx**	**MHC I**	**MHC IIa**	**MHC IIx**
Training group (n=16)	29.1 ± 13.3	52.6 ± 12.2[#]	18.1 ± 11.4[#]	29.4 ±10.9	59.4 ± 11.6	11.1 ± 9.1*
Control group (n=6)	22.8 ±14.9	70.2 ± 17.2	6.8 ± 10.9	25.8 ± 21.0	67.3 ± 20.6	7.0 ±13.0

* $p < 0.05$ in comparison with pre-training period, [#] $p < 0.05$ in comparison with control group.

2.4. Changes in MLC isoform composition

During the training period, the proportion of MLC1f changed from 23.9 ± 3.8% to 25.5 ± 2.0% and the proportion of MLC2f changed from 17.8 ± 5.0% to 18.8 ± 5.4% in the training group. None of the changes in MLC isoforms reached statistical significance (Table 6). In the training group, the changes of MLC1f isoform correlated negatively with those of MLC1s isoform ($r = –0.79$; $p < 0.05$), and the changes of MLC1s isoform correlated positively with changes of MHC I isoform ($r = 0.81$; $p < 0.05$) and negatively with changes in maximal anaerobic cycling power ($r = –0.81$; $p < 0.05$).

Table 6. Proportion of myosin light chain (MLC) isoforms in the *vastus lateralis* muscle before and after 54-week strength and power training program in training group subjects (n = 8). Values are means (± SD)

	MLC1s	**MLC1f**	**MLC2s**	**MLC2f**	**MLC3**
Before	32.0 ± 5.4	23.9 ± 3.8	13.6 ± 3.8	17.8 ± 5.0	12.7 ± 5.2
After	30.6 ± 4.4	25.5 ± 2.0	13.7 ± 2.9	18.8 ± 5.4	11.3 ± 4.6

[1] s = slow type, f = fast type

3. Adaptive changes of myosin heavy and light chain isoforms in single muscle fibers

3.1. Changes of MHC profile in single muscle fibers

The MHC expression, analysed by using single fibers, was determined for a total of 921 fibers (456 before and 465 after the training period). According to the MHC isoform expression, six distinct fiber types were separated; three pure and six hybrid type fibers (Fig. 7). We observed one extra band which migrated on gel matrix to the slow type MHC region (Fig. 2) but more slowly than MHC I (Fig. 7, lane 3). The fibers containing MHC I and yet unknown MHC isoforms were marked as unidentified hybrid fibers (MHC I/unidentified). Individual results and group means of the MHC isoform expressions analysed in single muscle fibers are presented in Table 7.

The number of pure slow type fibers (MHC I), which are well expressed in baseline, decreased during the training period in all participants (n = 5): totally from 243/456 (53.3%) to 188/465 (40.4%). The sum for the numbers of fibers expressing MHC I (types MHC I + MHC I/unidentified + MHC I/IIa + MHC IIa/I) resulted in 267 fibers, i.e., 58.5% of all observed fibers before the intervention and 222 fibers, i.e. 47.7% after the training period. Decrease in the number of fibers expressing MHC I either in pure type or hybrid type fibers was similar (12.9 and 10.8%-units, respectively). Contrary to this, the number of pure type fibers of MHC IIa increased in all participants (excluding subject nr. 5; Table 7) from 147 (32.2%) to 199 (42.8%). By summing the numbers of all fibers expressing MHC IIa (MHC I/IIa + MHC IIa/I + MHC IIa + MHC IIa/x + MHC IIax + MHC IIx/a) we obtained a total of 189 fibers (41.4%) before and 248 fibers (53.3%) after the training period. Correspondingly, increase of both pure type and hybrid type MHC IIa fibers was simultaneous and of same size as decreases of slow MHC fibers (10.6 and 11.9%-units, respectively).

The pure fibers expressing MHC IIx appeared only in two participants before the training period, three fibers in both participants (totalling 1.3%). After the training period, we observed fibers expressing MHC IIx in four participants, totalling 5 fibers (1.1%). A sum of the numbers of fibers containing the MHC IIx (MHC IIa/x + MHC IIax + MHC IIx/a + MHC IIx) totalled 42 fibers (9.2%) before and 44 fibers (9.4%) after the training period (Table 7).

The hybrid fibers were observed in all participants. The total amount of hybrid fibers was 60 fibers (13.1%) before and 73 fibers (15.8%) after the training period. The number of slow type hybrid fibers (co-expressed with MHC I and MHC IIa) increased from 6 (1.3%) to 10 (2.2%) during the training period. A minority of hybrid fibers, 18 fibers (3.9%) before and 24 fibers (5.2%) after the training period was co-expressed with MHC I and presently unidentified MHC isoform (termed as type MHC I/unidentified). All these fibers were highly dominated by MHC I isoform. The fast type hybrid fibers co-

expressing with MHC IIa and MHC IIx totalled 36 fibers (7.9%) before and 39 fibers (8.4%) after the training period.

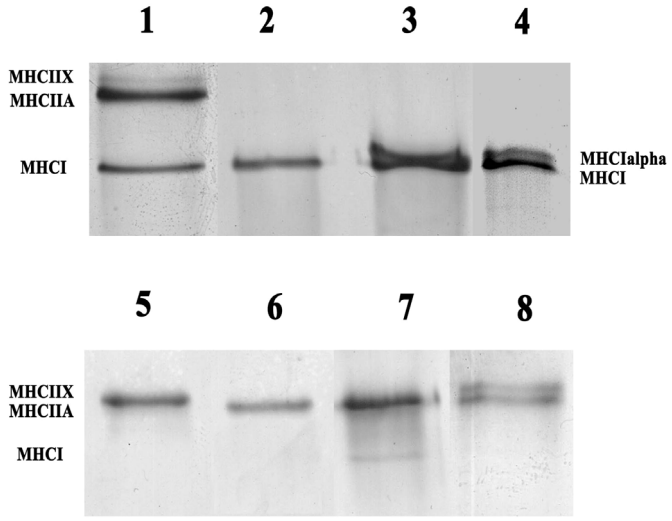


Figure 7. Example of 7.2% SDS-PAGE separation of MHC isoforms in muscle sample homogenate and in single muscle fibers. Lane 1, muscle sample homogenate; lane 2, MHC I type fiber; lane 3, unidentified MHC profile; lane 4, marker mixture of rat Soleus + cardiac muscle; lane 5, MHC IIx type fiber; lane 6, MHC IIa type fiber; lane 7, MHC IIa/I type fiber; lane 8, MHC IIax type fiber

3.2. Co expression of MHC and MLC in single muscle fibers

The data of MLC isoforms presented for fibers expressing distinct MHC isoforms are shown in Table 8. All pure fibers expressing MHC I appeared with MLC 1s and 2s isoforms both before and after the training period. More than half of the fibers expressing MHC I isoform also contained MLC 1f isoform: the MLC 1f was expressed in 60.6% of fibers before and in 57.1% of fibers after the training period (Table 8). Further, MLC 2f was expressed in 21.2% of pure fibers expressing MHC I isoforms before and in 28.6% of the corresponding fibers after the training period. All pure types of fibers expressing MHC IIa or MHC IIx contained MLC 1f isoform, but some of them also contained slow types of MLC isoforms. For example, 15 (26.3%) from 57 fibers expressing MHC IIa contained MLC 1s, 57 fibers (100%) contained MLC 1f, 51 fibers (89.5%) contained MLC 2f, 6 fibers (10.5%) contained MLC 2s and 6 fibers (10.5%) contained MLC 3 isoforms before the training period (Table 8). MLC 3 isoform appeared in all fibers expressing main MHC isoforms and seemed to appear more frequently after the training period than before it in the fibers expressing MHC I and MHC IIa (Table 8).

Table 7. The expression of MLC isoforms before and after power-type strength training in single muscle fibers typed according to MHC isoforms

Fiber type	MHC I		MHC I/unidentif		MHC I/IIa		MHC IIa/I		MHC IIa		MHC IIa/x		MHC IIax		MHC IIx/a		MHC IIx	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
MLC 1s	99	84	14	12	1	3	0	1	15	51	6	2	0	2	2	2	2	1
MLC 2s	99	84	14	12	0	3	0	0	6	21	3	0	0	2	2	0	2	1
MLC 1f	60	48	6	10	1	2	0	3	57	90	12	6	12	6	0	8	4	4
MLC 2f	21	24	0	0	1	3	0	3	51	87	9	6	12	6	0	8	2	3
MLC 3	9	21	0	0	1	1	0	3	6	21	3	2	8	4	2	0	2	4
nr of fibers	99	84	14	12	1	3	0	3	57	90	12	6	12	8	2	8	4	4

Table 8. The expression of MLC isoforms before and after power-type strength training in single muscle fibers typed according to MHC isoforms

Fiber type	MHC I		MHC I/unidentif		MHC I/IIa		MHC IIa/I		MHC IIa		MHC IIa/x		MHC IIax		MHC IIx/a		MHC IIx	
	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
MLC 1s	99	84	14	12	1	3	0	1	15	51	6	2	0	2	2	2	2	1
MLC 2s	99	84	14	12	0	3	0	0	6	21	3	0	0	2	2	0	2	1
MLC 1f	60	48	6	10	1	2	0	3	57	90	12	6	12	6	0	8	4	4
MLC 2f	21	24	0	0	1	3	0	3	51	87	9	6	12	6	0	8	2	3
MLC 3	9	21	0	0	1	1	0	3	6	21	3	2	8	4	2	0	2	4
nr of fibers	99	84	14	12	1	3	0	3	57	90	12	6	12	8	2	8	4	4

4. Changes in glucose metabolism and muscle fiber types in middle-aged subjects during long-term strength and power training

Weight reduction during the 2-year follow-up was 5.1 ± 6.2 kg ($p = 0.007$) in the IGT_{fast} group 3.6 ± 3.2 kg ($p = 0.003$) in the IGT_{slow} group. The difference between the groups was not statistically significant. At baseline, fasting glucose, 2-h glucose, 2-h insulin and HbA_{1c} were similar in the IGT_{slow} and IGT_{fast} groups, while fasting insulin ($p < 0.05$) and HOMA-IR (n.s.; $p = 0.087$) were higher in the IGT_{fast} group. During the 2-year intervention, fasting glucose, fasting insulin and HOMA-IR decreased significantly in the IGT_{fast} group, and 2-h glucose and HbA_{1c} decreased in both groups.

The intervention slightly decreased the GSK-3-β protein content ($p = 0.086$) in the *vastus lateralis* muscle and caused a significant decrease of the GSK-3-$\alpha\beta$ protein content ($p < 0.05$) in the IGT_{fast} group.

There were no significant changes in the amount of the Akt1/PKB protein in the IGT_{fast} group or in the IGT_{slow} group during the 2-year intervention.

Exercise training did not affect significantly the proportion of MHC I ($p = 0.096$) and and MHC IIx ($p = 0.105$) isoforms in the *vastus lateralis* muscle in IGT_{slow} group (Table 9).

Table 9. MHC profile at baseline and 2-year follow-up in subjects with impaired glucose tolerance (IGT), divided into the slow and fast type subgroups (mean ± SD)

	IGT_{SLOW} group (n = 11)		IGT_{FAST} group (n = 11)	
	Baseline	2-year follow-up	Baseline	2-year follow-up
MHC I	46.4 ± 9.0	49.2 ± 10.6	30.6 ± 3.6*	31.2 ± 5.9**
MHC IIa	36.9 ± 11.1	38.8 ± 4.6	47.1 ± 6.8**	47.9 ± 7.1**
MHC IIx	16.7 ± 10.5	12.0 ± 7.5	22.7 ± 7.4	20.9 ± 8.3**

*P < 0.01 between groups
**P < 0.001 between groups

DISCUSSION

1. The profile and distribution of MHC isoforms in middle-aged sedentary persons

The proportion of MHC isoforms of 89 middle-aged sedentary persons demonstrated large heterogeneity. For example, MHC I proportion varied from 9% to 74% in middle-aged sedentary participants. The large heterogeneity is very specific to human skeletal muscle, which has been repeatedly indicated in previous studies (Klittgaard *et al.*, 1990; Harridge *et al*., 1996; Andersen and Aagaard 2000; Esmarck *et al.*, 2001; Hostler *et al*., 2001; Campos *et al*., 2002; Nuhr *et al*., 2003). Even less data exists where slow or fast type MHC isoforms highly dominated in sedentary or untrained persons. The variation of MHC I and MHC IIa isoforms of 89 middle-aged sedentary persons demonstrated the normal distribution. For the first time the distribution of MHC isoforms in so many samples from human *vastus lateralis* muscle has been examined. In more than half of participants of MS group the MHC I proportion varied from 21 to 40%. The high proportion of MHC I isoform is characteristic to endurance athletes and fast type MHC isoforms are dominating in sprinters` and power type athletes` skeletal muscle (Ricoy *et al*., 1998; Andersen *et al*., 2000; Harber *et al*., 2000).

The comparison of MHC isoforms proportion between middle-aged and young participants did not show significant differences. The development of MHC I proportion has been indicated during aging and fast type MHC isoforms dominate in young adults. Previously it has been shown that age-related changes in muscle structure and function are more rapid after the age of 50 (Frontera *et al*., 1991; Lindle *et al*., 1997; Akima *et al*., 2001). The present study supports the previous notion – in middle-aged persons the age-related changes have not emerged yet.

2. MHC adaptation to exercise training

During the 54 weeks of strength and power training the maximal anaerobic cycling power improved in all sixteen subjects in the training group, and the mean maximal jumping height in the training group. These changes were in line with a tendency of increased proportion of MHC IIa and a decreased proportion of MHC IIx isoforms in muscle sample homogenate. Similar adaptive responses of MHC IIx to regular exercise training have been shown in previous studies (Sharman *et al*., 2001; Campos *et al*., 2002). Both endurance, and strength and power types of physical activity decrease the proportion of the fastest human MHC isoform (Klitgaard *et al.*, 1990; Andersen *et al*., 1994; Staron *et al*., 1994; Carroll *et al*., 1998; Tajsharghi *et al*., 2004). We found no studies in previous

literature where exercise training would have caused an increase in the proportion of MHC IIx isoform. The proportion of MHC IIx isoform only increases after declined neuromuscular activity, such as detraining (Andersen & Aagaard, 2000), or during inactivity, such as immobilization (Talmadge *et al*., 2002) or long-term bed rest (Andersen *et al*., 1999; Hostler *et al*., 2001; Trappe *et al*., 2004).

In the present study, the training lasted for 54 weeks and decreased the proportion of MHC IIx by 7.0%-units. Similar decreases of MHC IIx have been reported in other studies with shorter exercise interventions (Sharman *et al.,* 2001; Campos *et al*., 2002; Putman *et al*., 2004). In the study with young adults, only six weeks of exercise training (combined strength and endurance training) resulted in a significant decrease in MHC IIx content (Putman *et al*., 2004).

The increase of MHC IIa and decrease of MHC IIx isoforms have been suggested to reflect a transition from the fast to the slower type of MHC; this also emerged in the present study. A similar MHC transition has been found after heavy resistance training in young (Adams *et al*., 1993; Campos *et al*., 2002; Liu *et al*., 2003) and old subjects (Sharman *et al*., 2001). Alternative, bi-directional MHC transitions from MHC I and MHC IIx to MHC IIa have previously been demonstrated after sprint training (Andersen *et al*., 1994). In the present study, the mean percentage of MHC I remained the same during the training period, which was comparable with the results of previous studies with resistance or strength training interventions (Hostler *et al*., 2001; Campos *et al*., 2002).

3. Adaptive changes in muscle fibers

Although 54-week strength and power training affects MHC profile, significant changes in the composition of muscle fibers using traditional histochemical fiber typing were not observed. MHC profile analysis and histochemical fiber type analysis are not fully comparable. It has been shown that MHC profile responds faster to exercise stimulus than mATPase fiber profile does (Staron *et al*., 1994) and that, for example, part of histochemically assessed IIB fibers contain MHC IIa isoforms (Sant`ana Pereira *et al*., 1995). Thus, the MHC profile analyses are more sensitive measures to show changes in functional properties of muscle fibers as a result of exercise training than the mATPase fiber profile analyses.

4. MLC adaptation to exercise training

In the present study, strength and power training lasting 54 weeks tended to increase the proportion of MLC1f in the *vastus lateralis* muscle in the training group. In addition, the negative correlation between the changes of MLC1f and MLC1s proportions ($r = –0.79$; $p < 0.05$) shows that these alterations were in the same direction in most of the subjects (6/8). Trappe and co-workers (2000; 2001) studied the effects of a 12-week resistance training intervention on MLC isoform proportions in subjects aged approximately 74 years and found no significant changes in single muscle fibers of *m. vastus lateralis*. Their exercise program, however, differed from our program: the exercises were performed with heavy loads (80% of 1 RM) and with slow speed which resulted in increased cell size, strength and contractile velocity in both slow and fast MHC muscle fibers ('more pronounced in MHC I fibers') in men (Trappe *et al*., 2000) and only in MHC I muscle fibers in women (Trappe *et al*., 2001). In contrast, we used varying (30–85% of 1 RM) but mainly lower loads and high exercise tempo or high contractile velocity (power-type resistance training). Furthermore, the subjects in the study by Trappe *et al*. (2001) were older, and the training period was only a quarter of that in our study. Given that the duration of the training period in our study was much longer, that the subjects had rather high proportion of fast MHC muscle fibers in their *vastus lateralis* muscles, and that the training focused on the fast muscle fibers, the tentative changes in MLC isoforms can be assumed to be real. The small number of subjects and the fact that MHC and MLC fiber proportions were assessed using the muscle tissue homogenate instead of the single fiber method could be reasons for insufficient power of our study. Although the changes in the MLC isoforms during the long-term strength and power training were not statistically significant, they correlated with the changes in MHC profiles. The changes in MLC isoforms may possibly be associated with the transition of MHC isoforms as a result of the strength and power training and thus indicate improved muscle contraction. This, however, remains to be investigated in the future.

5. Role of training duration in adaptive changes of MHC isoforms

The subjects of the present study were advised to exercise 3 times a week according to a special strength and power training program for a period of 54 weeks. In previous studies, the shortest exercise period shown to affect the MHC isoform profile (results from the *triceps brachii* muscle) has been six weeks of strength training with maximum contractions and training 3 times a week (+17.3% in MHC IIa and –13.9% in MHC IIx isoforms) and with a combination of three types of training (once a week per type): strength training

with maximum contractions, ballistic exercises, and stretch-shortening movements (+15% in MHC IIa and –9% in MHC I isoforms) (Liu *et al.*, 2003b). The training effects can be detected earlier in the upper arm muscles than in the postural muscles, e.g. *vastus lateralis* muscle.

Recently, Kyröläinen and co-workers (2005) demonstrated that during a 15-week power training period, drop jump increased in young, recreationally active men but there were no significant changes in the MHC isoforms and muscle fiber proportions. This may be due to the high initial training status and the fact that the subjects continued their previous endurance-type sport activities (cycling, walking and ball games) for 6 hours a week on the average. In the present study, the strength and power training lasted for 54 weeks, but we only observed an increase of 6.8%-units in MHC IIa and a decrease of 7.0%-units in MHC IIx isoforms. In both studies, the subjects exercised 3 times a week, as targeted in our study. Strength training with maximum contraction causes stronger stress for skeletal muscles and leads to muscle hypertrophy, compared to the exercises with varying relative loads, carried out in our training program. The training was focused more on developing the speed of muscle contraction than increasing the maximal strength of muscles. It is also possible that the adaptive changes in skeletal muscle structure are not as extensive in middle-aged as in young participants, e.g. as a result from a smaller margin for MHC IIx to change due to lower baseline proportions (Short *et al*., 2005) and perhaps also a lower synthesis rate of MHC in the older people (Hasten *et al*., 2000). An additional reason for low training effects can be that the power type strength training was not carried out equally successfully by all the subjects. This suggestion was supported by the correlation coefficient of $r = 0.61$ between the amount of earlier recreational sports activity and the reduction of MHC IIx isoform, since the experienced subjects were more skilled and could perform exercises more effectively. However, in both studies jumping performance increased significantly, perhaps due to neural adaptations (Moritani and De Vries, 1980; Häkkinen *et al*., 1985), but changes in MHC profiles did not reach statistical significance. That can be interpreted as the type and/or amount of training being not suitable for the subjects and the training including numerous varying elements bearing influence on MHC profiles with opposite manner.

6. Adaptive changes of MHC isoforms in single muscle fibers

After the power-type strength training for 22 weeks, remarkable changes occurred in well expressed pure fibers: the proportion of MHC I fibers decreased and that of MHC IIa fibers increased.

In previous studies where muscle sample homogenate was used, the results indicated fast to slow MHC transition while the proportion of MHC IIa

increased and the proportion of MHC IIx decreased (Adams *et al*., 1993; Sharman *et al*., 2001; Campos *et al*., 2002; Liu *et al*., 2003a). We found only two previous studies showing the decrease of MHC I due to physical training. In muscle sample homogenate the proportion of MHC I decreased and concomitant MHC IIa increased after short duration strength training of young physically active men (Liu *et al*., 2003b). Andersen *et al*. (1994) indicated the decrease of MHC I fibers during bi-directional MHC transition after three months of intensive strength training in athletes. Differently from the present study, the participants of the above mentioned studies were physically active before the intervention. It is possible that relatively high proportion of MHC I fibers is one reason why their proportion of MHC I fibers decreased during the training period. At the baseline, the proportion of MHC I fibers was 53.3% and the proportion of all fibers expressing MHC I reached 58.5%. Relatively high proportion of MHC I (~52–75%) has been reported in athletes (Andersen *et al*., 1994; Harber *et al*., 2002), but in sedentary participants of previous studies the proportion of MHC I was lower (~32%) (Klitgaard *et al*., 1990). It is possible that physical training affects mainly the dominating fiber type, as has previously been concluded by Staron *et al*. (1994). Similarly to the present study, in a paper by Andersen *et al*. (1994) more than half of fibers represented MHC I profile and the training period decreased them. It has to be admitted that five participants in the present study is an inadequate number for generalizations but individual data indicated decreased proportion of MHC I fibers in all participants and in addition, MHC IIa fibers proportion increased in four of them.

7. Adaptive changes of MHC isoforms in hybrid fibers during exercise training

Before the study we assumed that the proportion of hybrid fibers of middle-aged untrained persons would be higher than the later analysis indicated. This assumption was supported by recent studies where MHC profile has been examined using SDS-PAGE combined with silver staining in single muscle fibers of young and old sedentary persons (Trappe *et al*., 2000; 2001; Williamson *et al*., 2000; 2001). Although hybrid fibers have been associated with fibers` transition (Bottinelli, 2001, Pette and Staron, 2001) only few previous papers had indicated changes in their proportion during exercise training (Trappe *et al*., 2001; Raue *et al*., 2005). In the present study we observed slow to fast transition of pure MHC type fibers but in slow hybrid fibers (MHC I/IIa) only minor changes occurred. The slow hybrid fibers were expressed in all participants but their total proportion reached only 1.3% before and 2.2% after the training period. The analysis of the relative MHC dominance by densitometry indicated no changes in MHC IIa dominant slow hybrid fibers and only slight increase in the total number of MHC I dominant MHC I/IIa fibers. The changes occurred

only in two participants. The decreased proportion of slow hybrid fibers has been recently demonstrated by Williamson *et al*. (2001) where the proportion of MHC I dominant MHC I/IIa fibers decreased during resistance training in young untrained men. In this study the proportion of MHC IIa fibers increased but MHC I fibers did not show any significant changes. These data indicate that the proportion of slow hybrid fibers had not altered due to changes in pure MHC I and MHC IIa fibers.

We observed fast hybrid fibers in all participants and the training period caused only small changes in their proportions. The relative MHC dominance by densitometry indicated the trend to increasing of nondominant MHC IIax fibers while in the number of MHC IIa dominant MHC IIa/x fibers emerged the trend towards decrease. The decreased proportion of fast hybrid fibers has been recently indicated during fast to slow MHC transition (decreased MHC IIx and increased MHC IIa) (Williamson *et al*., 2001). In our study we found very few fibers expressing only MHC IIx isoform both before and after the training period. Our data is in good accordance with previous investigations that human skeletal muscle contains only a small amount of pure MHC IIx fibers and the MHC IIx isoform is mainly expressed in hybrid fibers (Williamson *et al*., 2000; Raue *et al*., 2004).

A part of hybrid fibers contained MHC I and presently unidentified MHC band which migrated slightly more slowly than MHC I in gel matrix. We observed this type of fibers (termed as MHC I/unidentified) in the same three persons in both the pre- and post-training period and in one person after the training period. Until now, only cardiac muscle MHC Iα has been shown to migrate into this region (Hämälainen and Pette, 1997). The expression of MHC Iα has not been reported in human limb muscles but their expression has been established in masticatory muscles (Bredman *et al*., 1991; Stål *et al*., 1994; Korfage *et al*., 2001). The elevation of MHC Iα mRNA has been recently reported by Liu *et al*. (2003a) after strength training in limb muscle. Electrophoretical separation of MHC Iα complicated their low content and similar molecular mass with MHC I (McNally *et al*., 1989). We observed the expression of unidentified MHC isoform only in hybrid fibers in which dominated MHC I. In rabbit muscle fibers the proportion of additional slow MHC isoform reached 30% (Galler *et al*., 1997). It is possible that the protein content (especially of unidentified MHC isoform) of separated fibers exceeds the level of sensitivity of silver staining method. However, we do not exclude that the unknown MHC band in gel matrix of our study is an additional slow MHC isoform (possibly MHC Iα), but the identification of this needs an additional methodological control.

8. Adaptation of MLC in single muscle fibers during exercise training

Another original result in this study was in the large expression of mismatched MHC-MLC fibers, the proportion of which increased in MHC IIa fibers. The expression of MHC-MLC mismatched fibers in the present study is not in accordance with earlier data from Trappe *et al.* (2000, 2001). In their studies only matched MHC-MLC fibers' expression in human skeletal muscle has been indicated (Trappe *et al*., 2000, 2001). Although the term "matched/mismatched" MHC-MLC fiber was coined by Stephenson in 2001 (Stephenson, 2001), the expression of fast type MLC isoforms in slow (MHC I) fibers of human muscles has been indicated in previous studies as well (Larsson *et al*., 1993; Larsson *et al*., 1997).

Although the age difference of participants between the present study and the studies of Trappe *et al*. (2000, 2001) was large, it is very unlikely that these differences are conditioned by age-related changes. This discrepancy is rather caused by methodical differences. Differently from Trappe *et al*. (2000, 2001) we determined the slowest migrated MLC band in gel matrix as MLC 1s, similarly to the data of previous studies of human and animal muscles (Larsson *et al*., 1993, 1997; Bicer and Reiser, 2004).

We observed the expression of MLC 3 in all pure type fibers and their expression developed during the training period both in MHC I and MHC IIa fibers. The higher proportion of MLC 3 correlated with higher shortening velocity of muscle fibers (Larsson and Moss, 1997). In the present study the development of MLC 3 may be associated with jumping height and length, which improved during the training program. It is interesting that we did not observe the expression of MLC3 and MLC 2f isoforms in MHC I/unidentified fibers. The proportion of MLC3 in total MLC isoforms is small (~ 8–15%) (Trappe *et al*., 2001). It is possible that the low content of protein in samples limited the determination of MLC3 in some of the fibers. The higher concentration of proteins in fiber samples may help to detect MLC 3, but this protein concentration in our study was limited by size of fibers in muscle biopsy.

9. Role of types of muscle fibers and long-term exercise training on the glucose metabolism

A significant weight loss, resulting in improved insulin sensitivity and glucose tolerance, was achieved both in the IGT_{fast} and IGT_{slow} groups during the 2-year exercise and dietary intervention. The study design that we used does not allow us to reliably evaluate the separate effects of exercise or changes of dietary habits on weight loss on the improved glucose tolerance or skeletal muscle

glucose metabolism. This weakness prodceeds from our study design following, the Diabetes Prevention Study (Tuomilehto *et al*., 2001), which aimed at investigating the effects of lifestyle changes in preventing type 2 diabetes. Thus, we were not able to focus solely of the effects of exercise or diet. However, the role of exercise can be seen in the changes in skeletal muscle oxidative and glucolytic metabolism. Weight loss had no effect on the oxidative and glycolytic enzyme activities or muscle fiber composition (Gray *et al*., 2003), and it is known that resistance training decreases the mRNA and protein expression of the MHC IIx isoform in both young and elderly people (Sharman *et al*., 2001; Liu *et al*., 2003b). Comparison with controls would have strengthened our results significantly.

As far as the fiber-type composition is concerned, in the two subjects groups (IGT_{slow} and IGT_{fast}) no changes occured during the intervention period. However, exercise training slightly increased the proportion of MHC I and decreased the proportion of MHC IIx isoforms in the *vastus lateralis* muscle in IGT_{slow} group, indicating a shift towards more insulin-sensitive muscle fiber types. It is known that endurance type of exercise training results in an increase in the transition of fast glycolytic fibers to fast oxidative fibers, as well as an increase in capillary density. Type I and IIa fibers have greater capillary density, and they are more insulin sensitive and responsive than type IIb fibers. The difference between the proportions of MHC I and MHC II isoforms could partially explain why the IGT_{fast} subjects were more insulin-resistant than IGT_{slow} subjects at the baseline.

The present study raised a number of interesting questions which still remain open. In the future, studies should be designed with larger populations so that the effects of increased exercise and changes in dietary habits can be distinguished. Investigation of the long-term effects and the role of skeletal muscle fiber type, the dose and type of exercise and the mechanisms of improving the skeletal muscle glucose metabolism by diet or exercise, or by combining diet and exercise, would give valuable knowledge for further recommendations to improve the skeletal muscle metabolism in IGT subjects.

CONCLUSIONS

1. Normal distribution illustrated the percentage of MHC I and MHC IIa isoforms in middle-aged sedentary persons but significant differences of MHC isoforms proportion between young and middle-aged sedentary persons were not observed.
2. Long-term strength and power training improved results of anaerobic power and maximal jumping height, which correlated with the number of training sessions, but did not affect significantly the proportion of MHC and MLC isoforms in muscle sample homogenate of middle-aged sedentary persons.
3. In single muscle fibers, the proportion of MHC I fibers decreased and the proportion of MHC IIa fibers increased during the long-term power type strength training period.
4. The large number of mismatched MHC-MLC fibers was represented in skeletal muscle of middle-aged sedentary persons and their proportion elevated after the power type strength training in MHC IIa fibers.
5. Long-term resistance training improved glucose metabolism in middle-aged obese subjects but did not affect significantly the composition of MHC isoforms.

REFERENCES

Aagaard P., Andersen J.L. Correlation between contractile strength and myosin heavy chain isoform composition in human skeletal muscle. Med. Sci. Sport Exerc., 1998, 30(8): 1217–1222.

Adamo M.L., Farrar R.P. Resistance training and IGF involvement in the maintenance of muscle mass during aging process. Ageing Res. Rev. 2006, Aug 30.

Adams G., Hather B., Baldwin K., Dudley G. Skeletal muscle myosin heavy chain composition and resistance training. J. Appl. Physiol., 1993, 74: 911–915.

Akima H., Kano Y., Enomoto Y., Ishizu M., Okada M., Oishi Y., Katsuta S., Kuno S.Y. Muscle function in 164 men and women aged 20–84 yr. Med. Sci. Sports Exerc., 2001, 33(2): 220–226.

Andersen J.L., Aagaard P. Myosin heavy chain IIX overshoot in human skeletal muscle. Muscle & Nerve, 2000, 23: 1095–1104.

Andersen J.L., Gruschy-Knudsen T., Sandri C., Larsson L., Schiaffino S. Bed rest increases the amount of mismatched fibers in human skeletal muscles. J. Appl. Physiol., 1999, 86: 455–460.

Andersen J.L., Klitgaard H., Saltin B. Myosin heavy chain isoforms in single fibers from m. vastus lateralis of sprinters: influence of training. Acta Physiol. Scand., 1994, 151: 135–142.

Andersen J.L., Mohr T., Biering-Sørensen F., Galbo H., Kjær M. Myosin heavy chain isoform transformation in single fibers from M. Vastus lateralis in spinal cord injured individuals: effects of long-term functional electrical stimulation (FES). Pflügers Arch. – Eur. J. Physiol., 1996, 431: 513–518.

Andersen J.L., Schjerling P., Saltin B. Muscle, genes and athletic performance. Scient. Am., 2000, 289: 48–53.

Andersen R.E., Wadden T.A., Bartlett S.J., Zemel B., Verde T.J., Franckowiak S.C. Effects of lifestyle activity vs structured aerobic exercise in obese women: a randomized trial. JAMA., 1999, 27;281(4): 335–340.

Balagopal P., Ljungqvist O., Nair K.S. Skeletal muscle myosin heavy-chain synthesis rate in healthy humans. Am. J. Physiol. 1997, 272 (Endocrinol. Metab. 35): E45–E50.

Balagopal P., Schimke J.C., Ades P., Adey D., Nair K.S. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am. J. Physiol. Endocrinol. Metab., 2001, 280: E203–E208.

Bär A., Pette D. Three fast myosin heavy chains in adult rat skeletal muscle. FEBS Letters, 1988, 235: 153–155.

Berg H.E., Larsson L., Tesch P.A. Lower limb skeletal muscle function after 6 wk of bed rest. J. Appl. Physiol., 1997, 82(1): 182–188.

Benezra R., Davis R.L., Lockshon D., Turner D.L., Weintraub H. The protein Id: a negative regulator of helix-loop-helix DNA binding proteins. Cell, 1990, 61(1): 49–59.

Bicer S., Reiser S. Myosin light chain isoform expression among single mammalian skeletal muscle fibers: species variations. J. Muscle Res. Cell Motil., 2004, 25: 623–633.

Boldwin K.M., Haddad F. Plasticity in skeletal, cardiac, and smooth muscle. Invited review: Effect of different activity and inactivity paradigms on myosin heavy chain gene expression in straited muscle. J. Appl. Physiol., 2001, 90: 345–357.

Booth F.W., Gordon S.E., Carlson C.J., Hamilton M.T. Waging war on modern chronic diseases: primary prevention through exercise biology. J. Appl. Physiol., 2000, 88: 774–787.

Bosco C., Luhtanen P., Komi, P.V. A simple method for measurement of mechanical power in jumping. Eur. J. Appl. Physiol. Occup. Physiol., 1983, 50: 273–282.

Bottinelli R. Functional heterogeneity of mammalian single muscle fibers: do myosin isoforms tell the whole story? Pflügers Arch – Eur. J. Physiol., 2001, 443: 6–17.

Bottinelli R., Betto R., Schiaffino S, Reggiani C. Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibers. J. Physiol. (Lond.), 1994a, 478: 341–349.

Bottinelli R., Canepari M., Reggiani C., Stienen G.J. Myofibrillar ATPase activity during isometric contraction and isomyosin composition in rat skinned muscle fibers. J. Physiol. (Lond.), 1994b, 481: 663–675.

Bottinelli R., Reggiani C. Force-velocity properties and myosin light chain isoform composition of an identified type of skinned fibers from rat skeletal muscle. Pflügers Arch., 1995, 429: 592–594.

Bottinelli R., Reggiani C. Human skeletal muscle fibers: molecular and functional diversity. Progr. Biophys. & Molec. Biol., 2000, 73: 195–262.

Bottinelli R., Schiaffino S, Reggiani C. Force-velocity relationship and myosin heavy chain isoform composition of skinned fibers from rat skeletal muscle. J. Physiol. (Lond.), 1991, 437: 437–462.

Bredman J.J., Wessels A., Weijs W.A., Korfage J.A.M., Soffers C.A.S., Moorman A.F.M. Demonstration of "cardiac-specific" myosin heavy chain in masticatory muscles of human and rabbit. Histochem. J., 1991, 23: 160–170.

Brooke M.H., Kaiser K.K. Muscle fiber types: how many and what kind? Arch. Neurol., 1970, 23: 369–379.

Campos G.E.R., Luecke T.J., Wendeln H.K., Toma K., Hagerman F.C., Murray T.F., Ragg K.E., Ratamess N.A., Kraemer W.J., Staron R.S. Muscular adaptation in response to three different resistance-training regimens: specificity of repetition maximum training zones. Eur. J. Appl. Physiol., 2002, 88: 50–60.

Canepari M., Cappelli V., pellegrino M.A., Zanardi M.C., Reggiani C. Thyroid hormone regulation of MHC isoform composition and myofibrillar ATPase activity in rat skeletal muscles. Arch. Physiol. Biochem., 1998, 106: 308–315.

Carroll T.J., Abernethy P.J., Logan P.A., Barber M., McEniery M.T. Resistance training frequency: strength and myosin heavy chain responses to two and three bouts per week. Eur. J. Appl. Physiol. Occupat. Physiol., 1998, 78: 270–275.

D'Antona G., Pellegrino M.A., Adami R., Rossi R., Carlizzi C.N., Canepari M., Saltin B., Bottinelli R. The effect of ageing and immobilization on structure and function of human skeletal muscle fibers. J. Physiol., 2003, 552(2): 499–511.

DeFronzo R.A., Sherwin R.S., Kraemer N. Effect of physical training on insulin action and obesity. Diab., 1987, 36: 1379–1385.

Dishman R.K., Buckworth J. Increasing physical activity: a quantitative synthesis. Med. Sci. Sport Exerc., 1996, 28(6): 706–719.

Doherty T. J. Invited Review: Aging and sarcopenia. J. Appl. Physiol. 2003, 95, 1717–1727.
Dorrens J., Rennie M.J. Effects of ageing and human whole body and muscle protein turnover. Scand. J. Med. Sci. Sports, 2003, 13: 26–33.
Dutta C., Hadley E.C., Lexell J. Sarcopenia and physical performance in old age: Muscle & Nerve Suppl., 1997, 5: S5–S9.
Ennion S., Sant'Ana Pereira J., Sargeant A.J., Young A., Goldspink G. Characterization of human skeletal muscle fibers according to the myosin heavy chain they express. J. Muscle Res. Cell Motil., 1995, 16: 35–43.
Esmarck B., Andersen J.L., Olsen S., Richter E.A., Mizuno M., Kjær M. Timing of Postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. J. Physiol., 2001, 535(1): 301–311.
Frontera W., Hughes V.A., Lutz K.J., Evans W.J. A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. J. Appl. Physiol., 1991, 71: 644–650.
Gaiozzo V.J., Baker M.J., Baldwin K.M. Novel transitions in MHC isoforms: separate and combined effects of thyroid hormone and mechanical unloading. J. Appl. Physiol., 1998, 85: 2237–2248.
Gallagher P., Trappe S., Harber M., Creer A., Mazzetti S., Trappe T., Alkner B., Tesch P. Effects of 84-days of bedrest and resistance training on single muscle fiber myosin heavy chain distribution in human vastus lateralis and soleus muscles. Acta Physiol. Scand., 2005, 185: 61–69.
Galler S., Hilber K., Golsch B., Pette D. Two functionally distinct myosin heavy chain isoforms in slow skeletal muscle fibers. FEBS Letters, 1997, 410: 150–152.
Gastro M.J., Apple D.F., Staron R.S., Campos G.E.R., Dudley G.A. Influence of complete spinal cord injury on skeletal musle within 6 mo of injury. J. Appl. Physiol (Lond.), 1996, 491: 813–824.
Gerrits H.L., Hopman M.T.E., Offringa C., Engelen B.G.M., Sargeant A.J., Jones D.A., Haan A. Variability in fiber properties in paralysed human quadriceps muscles and effects of training. Pflügers Arch. – Eur. J. Physiol., 2003, 445: 734–740.
Golay A., DeFronzo R.A., Ferrannini E. et al. Oxidative and non-oxidative glucose metabolism in non-obese type 2 (non-insulin-dependent) diabetic patients. Diabetologia, 1988, 31: 585–591.
Goldspink G. Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. J. Anat., 1999, 194: 323–334.
Goldspink G., Yank S.Y. Effects of activity on growth factor expression. Int. J. Sport. Nutr. Exerc. Metab., 2001, 11 Suppl: S21–7. Review.
Greenlund L., Nair K. Sarcopenia – consequences, mechanism, and potential therapies. Mechan. Aging Devel., 2003, 124: 287–299.
Cross D.A., Alessi D.R., Cohen P., Andjelkovich M., Hemmings B.A. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature, 1995, 378: 785–789.
Häkkinen K., Kraemer W.J., Newton R.U., Alen M. Changes in electromyographic activity, muscle fiber and force production characteristics during heavy resistance/ power strength training in middle-aged and older men and women. Acta Physiol. Scand., 2001, 171: 51–62.

Hämälainen N., Pette D. Expression of an α-cardiac like myosin heavy chain in diaphragm, chronically stimulated, and denervated fast-twitch muscle of rabbit. J. Muscle Res. Cell Motil., 1997, 18: 401–411.

Halseth A.E., Bracy D.P., Wasserman D.H. Functional limitations to glucose uptake in muscles comprised of different fiber types. Am. J. Physiol. Endocrinol. Metab., 2001, 280: E994–E999.

Hameed M., Orrell R.W., Cobbold M., Goldspink G., Harridge S.D.R. Expression of IGF-l splice variants in young and old human skeletal muscle after high resistance exercise. J. Physiol., 2003, 547: 247–254.

Harber M.P., Fry A.C., Rubin M.R., Smith J.C., Weiss L.W. Skeletal muscle and hormonal adaptations to circuit weight training in untrained men. Scand. J. Med. Sci. in Sports**,** 2004, 14: 176–185.

Harber M.P., Gallagher P.M., Trautmann J., Trappe S.W. Myosin heavy chain composition of single muscle fibers in male distance runners. Int. J. Sports Med., 2002, 23: 484–488.

Harridge S.D.R., Bottinelli R., Canepari M., Pellegrino M.A., Reggiani C., Esbjörnsson M., Saltin B. Whole-muscle and single-fiber contractile properties and myosin heavy chain isoforms in humans. Pflügers Arch. – Eur. J. Physiol., 1996, 432: 913–920.

Hasten D.L., Pak-Loduca J., Obert K.A., Yarasheski K.E. Resistance exercise acutely increases MHC and mixed muscle protein synthesis rates in 78–84 and 23–32 yr olds. Am. J. Physiol. Endocrin. Metab., 2000, 278: E620–E626.

He J., Watkins S., Kelley D.E. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. Diabetes, 2001, 50: 817–823.

Henriksen E.J., Bourey R.E., Rodnick K.J., Koranyi L., Permutt M.A., Holloszy J.O. Glucose transport protein content and glucose transport capacity in rat skeletal muscles. Am. J. Physiol. Endocrinol. Metab. 1990, 259: E593–E598.

Hickey M.S., Weidner M.D., Gavigan K.E., Zheng D., Tyndall G.L., Houmard J.A. The insulin action–fiber type relationship in humans is muscle group specific. Am. J. Physiol. Endocrinol. Metab., 1995, 269: E150–E154.

Holloszy J.O., Kohrt W.M. Exercise. In: *Handbook of Physiology. Aging.* Bethesda, MD: Am. Physiol. Soc., 1995, Sect.11, Chapt 24, 633–666.

Hortobágyi T., Dempsey L., Fraser D., Zheng D., Hamilton G., Lambert J., Dohm L. Changes in muscle strength, muscle fiber size and myofibrillar gene expression after immobilization and retraining in humans. J. Physiol. (Lond.), 2002, 524: 293–304.

Hostler D., Schwirian C., Campos G., Toma K., Crill M., Hagerman G., Hagerman F., Staron R. Skeletal muscle adaptations in elastic resistance-trained young men and women. Eur. J. Appl. Physiol., 2001, 86: 112–118.

Hughes S.M., Chi M.M., Lowry O.H., Gundersen K. Myogenin induces a shift of enzyme activity from glycolytic to oxidative metabolism in muscles of transgenic mice. J. Cell Biol., 1999, 3,145(3): 633–642.

Ingalls C.P., Barnes W.S., Smith, S.B. Interaction between clenbuterol and run training: effects on exercise performance and MLC isoform content. J. Appl. Physiol., 1996, 80: 795–801.

Ingier F. Effects of endurance training on muscle fiber ATP-ase activity, capillary supply and mitochondrial content in man. J. Physiol., 1979, 294: 419–432.

Izquierdo M., Häkkinen K., Antón A., Garrues M., Ibañez J., Gorostiaga E. Maximal strength and power, endurance performance, and serum hormones in middle-aged and elderly men. Med. Sci. Sports Exerc., 2001, 33: 1577–1587.

Izquierdo M., Häkkinen K., Ibañez J., Garrues M., Antón A., Zúninga A., Larrion J.L., Gorostiaga E. Effects of strength training on muscle power and serum hormones in middle-aged and old men. J. Appl. Physiol., 2001, 90: 1497–1507.

Izquierdo M., Ibañez J., Gorostiaga E., Garrues M., Zuniga A., Antón A., Larrion J.L., Häkkinen K. Maximal strength and power characteristics in isometric and dynamic actions of the upper and lower extremities in middle-aged and older men. Acta Physiol. Scand., 2001, 167: 57–68.

James D.E., Jenkins A.B., Kraegen E.W. Heterogeneity of insulin action in individual muscles in vivo: euglycemic clamp studies in rats. Am. J. Physiol. Endocrinol. Metab., 1985, 248: E567–E574.

Janssen I., Katzmarzyk P.T., Boyce W.F., King M.A., Pickett W. Overweight and obesity in Canadan adolescents and their associations with dietary habits and physical activity. J. Adolesc. Health, 2004, 35(5): 360–367.

Klitgaard H., Bergman O., Betto R., Salviati G., Schiaffino S., Clausen T., Saltin B. Co-existence of myosin heavy chain I and IIa isoforms in human skeletal muscle fibers with endurance training. Pflügers Arch., 1990, 416: 470–472.

Klitgaard H., Zhou M., Richter E.A. Myosin heavy chain composition of single fibers from m. biceps brachii of male body builders. Acta Physiol. Scand., 1990, 140: 175–180.

Klitgaard H., Zhou M., Schiaffino S., Betto R., Salviati G., Saltin B. Ageing alters the myosin heavy chain composition of single fibers from human skeletal muscle. Acta Physiol. Scand., 1990, 140: 55–62.

Koistinen H.A., Zierath J.R. Regulation of glucose transport in human skeletal muscle. Ann. Med., 2002, 34(6): 410–418. Review.

Korfage J.A.M., Schueler Y.T., Brugman P., Van Eijden T.M.G.I. Differences in myosin heavy-chain composition between human jaw-closing muscles and supra- and infrahyoid muscle. Arc. Oral Biol., 2001, 46: 821–827.

Kyröläinen H., Avela J., McBride J.M., Koskinen S., Andersen J.L., Sipilä S., Takala T.E.S., Komi P.V. Effect of power training on muscle structure and neuromuscular performance. Scand. J. Med. Sci. in Sports, 2005, 15: 58–64.

Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970, 227: 680–686.

Larsson L., Moss R.L. Maximum velocity of shortening in relation to myosin isoform composition in single fibers from human skeletal muscles. J. Physiol. (Lond.), 1993, 472: 595–614.

Larsson L., Xiapeng L., Berg H.E., Frontera W.R. Effects of removal of weight-bearing function on contractility and myosin isoform composition in single human skeletal muscle cells. Pflügers Arch. – Eur. J. Physiol. 1996, 432: 320–328.

Larsson L., Xiapeng L., Frontera W.R. Effect of aging on shortening velocity and myosin isoform composition in single human skeletal muscle cells. Am. J. Physiol. 1997, 272: C638–C649.

Lexell J., Taylor C., Sjöstrom M. What is the cause of the aging atrophy? Total number, size and proportion of different fiber type studies in whole *vastus lateralis* muscle from 15- to 83-year-old men. J. Neurol. Sci., 1988, 84: 275–294.

Lindle R.S., Metter E.J., Lynch N.A., Fleg J.L., Fozard J.L., Tobin J., Roy T.A., Hurley B.F. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. J. Appl. Physiol., 1997, 83: 1581–1587.

Liu Y., Lormes W., Reissnecker S., Steinacker J.M. Effects of high intensity resistance and low intensity endurance training on myosin heavy chain isoform expression in highly trained rowers. Int. J. Sports Med., 2003a, 24: 264–270.

Liu Y., Schlumberger A., Wirth K., Schmidtbleicher D., Steinacker J.M. Different effects on human skeletal myosin heavy chain isoform expression: strength vs. combination training. J. Appl. Physiol. 2003b, 94: 2282–2288.

Lowey S., Waller G.S., Trybun K.M. Skeletal muscle myosin light chains are essential for physiological speed of shortening. Nature, 1993, 365: 454–456.

March D.R., Criswell D.S., Carson J.A., Booth F.W. Myogenic regulatory factors during regeneration of skeletal muscle in young, adult, and old rats. J. Appl. Physiol., 1997, 83(4): 1270–1275.

Mårin P., Andersson B., Krotkiewski M., Björntorp P. Muscle fiber composition and capillary density in women and men with NIDDM. Diab. Care, 1994, 17: 382–386.

Mcbride J., Häkkinen A., Häkkinen K., Kraemer W.J. Changes in myosin heavy chain composition with heavy resistance training in 60-to 75-year-old men and women. Eur. J. Appl. Physiol., 2001, 84: 127–132.

Mcnally E.M., Kraft R., Bravo-Zehnder M., Taylor D.A., Leinwald L.A. Full-length rat alpha and beta cardiac myosin heavy chain sequences. Comparisons suggest a molecular basis for functional differences. J. Mol. Biol., 1989, 210(3): 665–671.

Nikoulina S.E., Ciaraldi T.P., Mudaliar S., Carter L., Johnson K., Henry R.R. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. Diabetes, 2002, 51: 2190–2198.

Nikoulina S.E., Ciaraldi T.P., Mudaliar S., Mohideen P., Carter L., Henry R.R. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. Diabetes, 2000, 49: 263–271.

Nuhr M., Crevenna R., Cohlsch B., Bittner C., Pleiner J., Wiesinger G., Fialka-Moser V., Quittan M., Pette D. Functional and biochemical properties of chronically stimulated human skeletal muscle. Eur. J. Appl. Physiol., 2003, 89: 202–208.

Nuutila P., Koivisto V.A., Knuuti J., Ruotsalainen U., Teras M., Haaparanta M., Bergman J., Solin O., Voipio-Pulkki L.M., Wegelius U. *et al*. Glucose-free fatty acid cycle operates in human heart and skeletal muscle *in vivo*. J. Clin. Invest., 1992, 89(6): 1767–1774.

O'Neill D.S., Zheng D., Anderson W.K., Dohm G.L., Houmard J.A. Effect of endurance exercise on myosin heavy chain gene regulation in human skeletal muscle. Am. J. Physiol., 1999, 276: R414–R419.

Pehme A., Alev K., Kaasik P., Seene T. Age related changes in skeletal muscle myosin heavy chain composition: effect of mechanical loading. J. Ageing Phys. Activity, 2004, 11, 29–44.

Peter J.B., Barnard R.J., Edgerton V.R., Gillespie C.A., Stempel K.E. Metabolic profiles of three fiber types of skeletal muscle in guinea pig and rabbit. Biochem., 1972, 11: 2627–2633.

Pette D., Staron R.S. Transitions of muscle fiber phenotypic profiles. Histochem. Cell Biol., 2001, 115: 359–372.

Phillips S.M., Tipton K.D., Aarsland A., Wolfe S.E., Wolfe R.R. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am. J. Physiol. Endocrinol. Metab. 1997, 273: E99–E107.

Phillips S.M., Tipton K.D., Ferrando A.A., Wolfe R.R. Resistance training reduces the acute exercise-induced increase in muscle protein turnover. Am. J. Physiol. 1999, 276 (Endocrinol. Metab. 39): E118–E124.

Putman C.T., Xu X., Gillies E., MacLeon I.M., Bell, G.J. Effects of strength, endurance and combined training on myosin heavy chain content and fiber-type distribution in humans. Eur. J. Appl. Physiol., 2004, 92: 376–384.

Ragg K.E., Ratamess N.A., Kraemer W.J., Staron R.S. Muscular adaptation in response to three different resistance-training regimens: specificity of repetition maximum training zones. Eur. J. Appl. Physiol., 2002, 88: 50–60.

Raue U., Terpstra B., Williamson D.L., Gallagher P.M., Trappe S.W. Effects of short-term concentric vs. eccentric resistance training on single muscle fiber MHC distribution in human. Int. J. Sports Med., 2005, 26: 339–343.

Reichman H., Pette D. A comparative microphotometric study of succinate dehydrogenase activity levels in type I, IIA and IIB fibers of mammalian and human muscles. Histochem., 1982, 74: 27–41.

Richardson C.R., Kriska A.M., Lantz P.M., Huyward R.A. Physical activity and mortality across cardiovascular disease risk groups. Med. Sci. Sport Exerc., 2004, 36(11): 1923–1929.

Ricoy J.R., Encinas A.R., Cabello A., Madero S., Arenas J. Histochemical study of the vastus lateralis muscle fiber types of athletes. J. Physiol. & Biochem., 1989, 54: 41–47.

Rusko H., Nummela A., Mero A. A new method for the evaluation of anaerobic running power in athletes. Eur. J. Appl. Physiol. 1993, 66: 97–101.

Sant`ana Pereira, J.A.A., Wessels, A., Nijtmans, L., Moorman, A.F.M., and Sargeant, A.J. New method for the accurate characterization of single human muscle fibers demonstrated a relation between mATPase and MyHC expression in pure and hybrid fiber type. J. Muscle Res. Cell Mot., 1995,16: 21–34.

Schaub M.C., Hefti M.A., Zeulling R.A., Morano I. Modulation of contractility in human cardiac hypertrophy by myosin essential light chain isoforms. Cardiovasc. Res., 1998, 37: 381–404.

Scholl M. Physical activity in an ageing population. Scand. J. Med. Sci. Sports, 2003, 13: 63–69.

Sharman M.J., Newton R.U., Triplett-McBride T., McGuigan M.R.M., McBride J.M., Häkkinen A., Häkkinen K., Kraemer W.J. Changes in myosin heavy chain composition with heavy resistance training in 60- to 75-year-old men and women. Eur. J. Appl. Physiol.**,** 2001, 84: 127–132.

Sharman M., Newton R., Triplett-McBride T., McGuigan M., McBride J., Häkkinen A., Häkkinen K., Kraemer W. Changes in myosin heavy chain composition with heavy resistance training in 60-to 75-year-old men and women. Eur. J. Appl. Physiol., 2001, 84: 127–132.

Short K.R., Vittone J.L., Bigelow M.L., Proctor D.N., Coenen-Schimke J.M., Rys P., Nair K.S. Changes in myosin heavy chain mRNA and protein expression in human skeletal muscle with age and endurance exercise training. J. Appl. Physiol., 2005, 99: 95–102.

Short K.R., Vittone J.L., Bigelow M.L., Proctor D.N., Nair K.S. Age and aerobic exercise training effects on whole body and muscle protein metabolism. Am. J. Physiol. Endocrinol. Metab., 2004, 286: E92–E101.

Simonaeu J.A., Bouchard C. Genetic determinism of fiber type proportion in human skeletal muscle. FASEB, 1995, 9: 1091–1095.

Simpson R.W., Shaw J.E., Zimmet P.Z. The prevention of type 2 diabetes – lifestyle change or pharmacotherapy? A challenge for the 21st century. Diab. Res. Clin. Pract., 2003, 59(3): 165–180.

Stål P., Eriksson P.-O., Schiaffino S., Butler-Browne G.S., Thornell L.-E. Differences in myosin composition between human oro-facial, masticatory, and limb muscles: enzyme-, immunochisto- and biochemical studies. J. Muscle Res. Cell Motil., 1994, 15: 517–534.

Staron R.S. Human skeletal muscle fiber types: delineation, development, and distribution. Can. J. Appl. Physiol., 1997, 22: 307–327.

Staron R., Hikida R., Hagerman F., Dudley G., Murray T. Human skeletal muscle fiber type adaptability to various workloads. J. Histochem. Cytochem., 1984, 32: 146–152.

Staron R.S., Karapondo D.L., Kraemer W.J., Fry A.C., Gordon S.E., Falkel J.E., Hagerman F.C., Hikida R.S. Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. J. Appl. Physiol., 1994, 76: 1247–1255.

Strandberg A., Strandberg T.E., Salomaa V.V., Pitkala K., Happola O., Miettinen T.A. A follow-up study found that cardiovascular risk in middle age predicted mortality and quality of life in old age. J. Clin. Epidemiol., 2004, 57(4): 415–421.

Stephenson G.M.M. Hybrid skeletal muscle fibers: a rare or common phenomenon? Clin. Exp. Pharmacol. Physiol., 2001, 28(8): 692–702.

Sugiura T., Murakami N. Separation of myosin heavy chain isoforms in rat skeletal muscle by gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Biomed. Res., 1990, 11: 87–91.

Surakka J., Alanen E., Aunola S., Karppi S.-L., Lehto P. Adherence to a power-type strength training programme in sedentary, middle-aged men and women. Adv. in Physioth., 2004, 6(3): 99–109.

Surakka J., Aunola S., Nordblad T., Karppi S.-L., Alanen E. Feasibility of power-type strength training for middle-aged men and women: self perception, musculoskeletal symptoms, and injury rates. Br. J. Sports Med., 2003, 37: 131–136.

Tajsharghi H., Stibrant Sunnerhagen K., Darin N., Kyllerman M., Oldfors A. Induced shift in myosin heavy chain expression in myosin myopathy by endurance training. J. Neurol., 2004, 251: 179–183.

Talmadge R.J. Myosin heavy chain isoform expression following reduced neuromuscular activity: potential regulatory mechanisms. Muscle & Nerve, 2000, 23: 661–679.

Talmadge R., Castro M., Apple D., Dudley G. Phenotypic adaptations in human muscle fibers 6 and 24 wk after spinal cord injury. J. Appl. Physiol., 2002, 92, 147–154.

Tamaki T., Uchiyama S., Uchiyama M., Akatsuka A., Yoshimura S., Roy R.R., Edgerton V.R. Limited myogenic response to a single bout of weight-lifting exercise in old rats. Am. J. Physiol. Cell Physiol., 2000, 278(6): C1143–52.

Tanner C.J., Barakat H.A., Dohm G.L. et al. Muscle fiber type is associated with obesity and weight loss. Am. J. Physiol. Endocrinol. Metab., 2002, 282: E1191–E1196.

Thorburn A.W., Gumbiner B., Bulacan F., Wallace P., Henry R.R. Intracellular glucose oxidation and glycogen synthase activity are reduced in non-insulin-dependent (type II) diabetes independent of impaired glucose uptake. J. Clin. Invest., 1990, 85: 522–529.

Trappe S., Godard M., Gallagher P., Carroll C., Rowden G., Porter D. Resistance training improves single muscle fiber contractile function in older women. Am. J. Physiol., Cell Physiol., 2001, 281: C398–C406.

Trappe S., Trappe T., Gallagher P., Harber M., Alkner B., Tesch P. Human single muscle fiber function with 84 day bed-rest and resistance exercise. J. Physiol., 2004, 557 (Pt2): 501–513.

Trappe, S., Williamson, D., Godard, M., Porter, D., Rowden, G., and Costill, D. Effect of resistance training on single muscle fiber contractile function in older men. J. Appl. Physiol., 2000, 89:143–152.

Vestergaard H., Lund S., Larsen F.S., Bjerrum O.J., Pedersen O. Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin resistant patients with non-insulin-dependent diabetes mellitus. J. Clin. Invest., 1993, 91: 2342–2350.

Wada M., Inashima S., Yamada T., Matsunaga S. Endurance training-induced changes in alkali light chain patterns in type IIB fibers of the rat. J. Appl. Physiol., 2003, 94: 923–929.

Wahrmann J.P., Winand R., Rieu M. Plasticity of skeletal myosin in endurance-trained rats (I). A quantitative study. Eur. J. Appl. Physiol., 2001, 84: 367–372.

Weiss S., Schiaffino S., Leinwald L.A. Comparative sequence analysis of the complete human sarcomeric myosin heavy chain family: implications for functional diversity. J. Mol. Biol. 1999, 290: 61–75.

Wickelgren I. New populations old add to poor nations' burdens. Sci., 1996, 273: 46–50.

Widrick J.J., Stelzer J.E., Shoepa T., Garner D.P. Functional properties of human muscle fibers after short-term resistance exercise training. Am. J. Physiol., 2002, 283: R408–R416.

Williamson D.L., Gallagher P.M., Carrol C.C., Raue U., Trappe S.W. Reduction in hybrid single muscle fiber proportions with resistance training. J. Appl. Physiol., 2001, 91: 1955–1961.

Williamson D.L., Godard M.P., Porter D.A., Costill D., Trappe S.W. Progressive resistance training reduces myosin heavy chain coexpression in single muscle fibers from older men. J. Appl. Physiol., 2000, 88: 627–33.

Willoughby D.S., Nelson M.J. Myosin heavy-chain mRNA expression after a single session of heavy-resistance exercise. Med. Sci. Sports Exerc., 2002, 34(8): 1262–1269.

Yarasheski K.E., Zachwieja J.J., Bier D.M. Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. Am. J. Physiol. Endocrinol. Metab., 1993, 265: E210–E214.

SUMMARY IN ESTONIAN

Pikaajalise jõutreeningu mõjul toimuvad adaptatiivsed muutused müosiini isovormilises kompositsioonis keskealiste inimeste skeletilihases

Arenenud Euroopa riikides on oluliselt suurenenud kesk- ja vanemaealise elanikkonna osakaal ning vähenenud inimeste kehaline aktiivsus. Vananemist ja vähest kehalist aktiivsust seostatakse skeletilihases eelkõige kiirete motoorsete ühikute kadumise ning lihaskiudude atroofiaga. Sellest tulenevalt on üha aktuaalsemaks muutunud võimaluste leidmine vananemisega kaasnevate muutuste ennetamiseks ning seeläbi inimese aktiivse eluea ning enesega toimetuleku perioodi pikendamiseks vanaduses.

Käesoleva töö eesmärgiks oli uurida pikaajalise jõutreeningu mõjul toimuvaid adaptatiivseid muutuseid müosiini raske- (MHC) ja kerge ahela (MLC) isovormilises kompositsioonis keskealiste inimeste skeletilihases.

Töö tulemustest nähtub, et erinevalt MHC IIx suhtelisest sisaldusest, vastas MHC I ja MHC IIa isovormi suhteline sisaldus keskealistel, istuva eluviisiga vaatlusaluste grupil (n = 89) normaaljaotusele. 54-nädalane jõutreening parandas vaatlusaluste (n = 9) anaeroobset võimekust ning maksimaalset üleshüppe kõrgust, kuid statistiliselt olulist muutust lihaskiudude kompositsioonis ja MLC isovormilises kompositsioonis ei täheldatud. Oluliselt langes 54-nädalase jõutreeningu järgel MHC IIx isovormi osakaal. Keskealistel II tüüpi diabeedi kahtlusega ülekaalulistel patsientidel (n = 22) langes kaheaastase vaatlusperioodi jooksul (regulaarne kehaline aktiivsus + toitumise jälgimine) kehakaal ning paranes süsivesikute ainevahetus, kuid olulisi muutuseid MHC isovormilises kompositsioonis ei ilmnenud.

Adaptatiivseid muutuseid MHC isovormilises kompositsioonis üksiku lihaskiu tasemel hinnati 921 isoleeritud lihaskius, 456 lihaskius enne ja 465 lihaskius pärast 22-nädalast jõutreeningut. Treeningu mõjul langes MHC I tüüpi lihaskiudude proportsioon kõigil vaatlusalustel (n = 5) ning MHC IIa tüüpi lihaskiudude hulk neljal vaatlusalusel. Ainult 11 eraldatud lihaskiudu (6 enne ja 5 pärast treeningperioodi) sisaldas MHC IIx isovormi.

Isoleeritud lihaskius MLC isovormilises kompositsioonis ilmnes suur heterogeensus. 22-nädalase jõutreeningu järgselt suurenes MHC-MLC hübriidkiudude arv MHC IIa tüüpi lihaskiududes ning MLC 3 ekspressioon MHC I ja MHC IIa tüüpi lihaskiududes.

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Raivo Puhke

PUBLICATIONS

Puhke R., Aunola S., Surakka J., Venojärvi M., Alev K., Seene T., Rusko H. The profile and distribution of myosin heavy chain isoforms in middle-aged sedentary persons. Journal of Sports Medicine and Physical Fitness, 2006, 46: 176–182.

Puhke R., Aunola S., Ailanto P., Alev K., Venojärvi M., Rusko H., Seene T. Adaptive changes of myosin isoforms in response to long-term strength and power training in middle-aged men. Journal of Sports Science and Medicine, 2006, 5: 349–358.

Venojärvi M., Puhke R., Hämäläinen H., Marniemi J., Rastas M., Rusko H., Nuutila P., Hänninen O., Aunola S. Role of skeletal muscle-fibre regulation glucose metabolism in middle-aged subjects with impaired glucose tolerance during a long-term exercise and dietary intervention. Diabetes, Obesity and Metabolism, 2005, Nov 7(6): 745–754.

CURRICULUM VITAE

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Education

1973–1984	Secondary School of Tapa
1987–1991	University of Tartu, Faculty of Exercise and Sport Sciences
1991–1993	MSc student at University of Tartu, Faculty of Exercise and Sport Sciences
1993	MSc "The effect of endurance training with different character on the content of myofibrillar proteins and their ratio"
1993–1997	PhD student at the Institute of Exercise Biology

Professional Emploiment

1994–1997	University of Tartu, Faculty of Exercise and Sport Sciences, Institute of Sport Training, researcher
Since 1997	University of Tartu, Institute of Exercise Biology and Physiotherapy, researcher

Specialized Courses

1996	CIMO grant to study at University of Jyväskylä
1997	CIMO grant to study at University of Jyväskylä

Membership in professional organizations

member of the Federation of European Physiological Societie

Scientific activity

Research field: The effect of physical activity for composition of skeletal muscle fibres, myofibrillar proteins and their isoformic composition

The total number of scientific publications is 32, including 5 articles in international peer-reviewed journals.

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Haridus

1973–1984	Tapa Keskkool
1987–1991	Tartu Ülikool, kehakultuuriteaduskond
1991–1993	magistrantuur Tartu Ülikool, kehakultuuriteaduskond, spordibioloogia instituut
1993	sporditeaduste magister, magistritöö teema "Erineva iseloomuga vastupidavustreeningu mõju skeletilihaste müofibrillaarvalkude sisaldusele ja omavahelistele suhetele"
1993–1999	doktorantuur Tartu Ülikooli spordibioloogia instituudi, funktsionaalse morfoloogia õppetoolis

Erialane teenistuskäik

1994–1997	Tartu Ülikool kehakultuuriteaduskond, treeningõpetuse instituut, teadur
1997–k.a	Tartu Ülikool kehakultuuriteaduskond, spordibioloogia ja füsioteraapia instituut, funktsionaalse morfoloogia õppetool, teadur

Erialane enesetäiendus

1996	CIMO grant, Jyväskylä Ülikool
1997	CIMO grant, Jyväskylä Ülikool

Erialaorganisatsioonid

Eesti Füsioloogia Seltsi liige

Teadustegevus

Peamised uurimisvaldkonnad: kehalise aktiivsuse mõju skeletilihaskiudude kompositsioonile, müofibrillaarvalkude sisaldusele ning nende isovormilisele kompositsioonile

Kokku on ilmunud 32 teaduspublikatsiooni, sh 5 artiklit rahvusvahelistes eelrefereeritavates ajakirjades ja kogumikes.